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(54) Title: FUSED 2,4-PYRIMIDINEDIONE COMBINATORIAL LIBRARIES AND BIOLOGICALLY ACTIVE FUSED 2,4-PYRAMIDINEDIONES

(57) Abstract

The invention provides chemistry libraries containing fused 2,4-pyrimidinediones. The invention also provides methods for the construction of fused 2,4-pyrimidinedione containing libraries. The invention further provides methods for the identification of bioactive, fused 2,4-pyrimidinediones from those libraries. The invention is still further directed to bioactive fused 2,4-pyrimidinediones.

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FUSED 2,4-PYRIMIDINEDIONE COMBINATORIAL LIBRARIES AND BIOLOGICALLY ACTIVE FUSED 2,4-PYRIMIDINEDIONES

TECHNICAL FIELD

This invention is directed to combinatorial chemistry libraries containing fused 2,4-pyrimidinediones. This invention is also directed to methods for constructing combinatorial chemistry libraries containing fused 2,4-pyrimidinediones. This invention is further directed to methods for the identification of bioactive fused 2,4-pyrimidinediones. This invention is still further directed to bioactive fused 2,4-pyrimidinediones.

BACKGROUND ART

Throughout this application, various publications, patents, and published patent applications are referred to by an identifying citation. The disclosures of the publications, patents, and published patent specifications referenced in this application are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

Modern day drug discovery is a multi-faceted endeavor. Researchers commonly delineate a biochemical pathway that is operative in a targeted pathological process. This pathway is analyzed with an eye toward determining its crucial elements: those enzymes or receptors that, if modulated, could inhibit the pathological process. An assay is constructed such that the ability of the important enzyme or receptor to function can be measured. The assay is then performed in the presence of a variety of molecules. If one of the assayed molecules modulates the enzyme or receptor in a desirable fashion, this molecule may be used directly in a pharmaceutical preparation or can be chemically modified in an attempt to augment its beneficial activity. The modified molecule that exhibits the best profile of beneficial activity may ultimately be formulated as a drug for the treatment of the targeted pathological process.

With the use of high-throughput screening techniques, one can assay the activity of tens of thousands of molecules per week. Where molecules can only be synthesized one at a time, the rate of molecule submission to an assay becomes a debilitating, limiting factor. This problem has led researchers to develop methods by which large numbers of molecules possessing diverse chemical structures can be rapidly and efficiently synthesized. One such method is the construction of chemical combinatorial libraries.

Chemical combinatorial libraries are diverse collections of molecular compounds. Gordon et al. (1995) Acc. Chem. Res. 29:144-154. These compounds are formed using a multistep synthetic route, wherein a series of different chemical modules can be inserted at any particular step in the route. By performing the synthetic route multiple times in parallel, each possible permutation of the chemical modules can be constructed. The result is the rapid synthesis of hundreds, thousands, or even millions of different structures within a chemical class.

For several reasons the initial work in combinatorial library construction focused on peptide synthesis. Furka et al. (1991) Int. J. Peptide Protein Res. 37:487-493; Houghton et al. (1985) Proc. Natl. Acad. Sci. USA 82:5131-5135; Geysen et al. (1984) Proc. Natl. Acad. Sci. USA 81:3998-4002; and Fodor et al. (1991) Science 251:767. The rapid synthesis of discrete chemical entities is enhanced where the need to purify synthetic intermediates is minimized or eliminated; synthesis on a solid support serves this function. Construction of peptides on a solid support is well-known and well-documented. Obtaining a large number of structurally diverse molecules through combinatorial synthesis is furthered where many different chemical modules are readily available; hundreds of amino acid modules are commercially available. Finally, many peptides are biologically active, making them interesting as a class to the pharmaceutical industry.

The scope of combinatorial chemistry libraries has recently been expanded beyond peptide synthesis. Polycarbamate and *N*-substituted glycine libraries have been synthesized in an attempt to produce libraries containing chemical entities that are similar to peptides in structure, but possess enhanced proteolytic stability, absorption and pharmacokinetic properties. Cho et al. (1993) Science 261:1303-1305; Simon et al. (1992) Proc. Natl. Acad. Sci. USA 89:9367-9371. Furthermore, benzodiazepine, pyrrolidine, and diketopiperazine libraries have been synthesized, expanding combinatorial chemistry to include heterocyclic entities. Bunin et al. (1992) J. Am. Chem. Soc. 114:10997-10998;

Murphy et al. (1995) J. Am. Chem. Soc. 117:7029-7030; and Gordon et al. (1995) Biorg. Medicinal Chem. Lett. 5:47-50.

Pyrimidinediones are a class of bioactive, heterocyclic molecules that have attracted considerable attention in the pharmaceutical industry. The benzo derivatives of this series (2,4-quinazolinediones) are represented as anti-inflammatory agents, analgesics, anticonvulsants, CNS agents, serotonin uptake inhibitors, antihypertensive agents, cardiovascular agents, and fungicides. Maillard et al. (1968) Fr. Chim. Ther. 3:100-106; Montginoul et al. (1988) Ann. Pharm. Franc. 46:223-232; Lowe et al. (1991) J. Med. Chem. 34:624-628; Mignani et al. (1993) Bioorg. Med. Chem. Lett. 3:1913-1918; Can. Pat. Appl. CA 2053475; Eur. Pat. Appl. EP 481342; and Smith et al. (1996) Bioorg. Med. Chem. Lett. 6:1483-1486. Naturally occurring bioactive compounds, such as theophylline and theobromine, that have found application as cardiotonic agents, broncholytic agents, vasodilators, psychotonic agents, and circulation analeptic agents are heterocyclic ring fused 2-4-pyrimidinediones. Roth et al. In: *Pharmaceutical Chemistry. Volume 1: Drug Synthesis* (1988) John Wiley & Sons. Recently, synthetic heterocyclic fused 2,4-pyrimidinediones, such as thieno and furopyrimidine-2,4-diones have been patented as serotonin antagonists and alpha adrenergic blocking agents. U.S. Patent No. 4,835,157.

Methods for the solution phase preparation of fused 2,4-pyrimidinediones have been reported. For example, the solution phase synthesis of pyridopyrimidinediones and quinazolinediones by the reaction, respectively, of an aminonicotinic ester or anthranilate derivative with isocyanate has been described. Lowe et al. (1991) J. Med. Chem. 34:624-628. The reaction of a carbomethoxyphenyl isocyanate with an amino acid is a further described example of solution phase chemistry used to construct fused pyrimidinediones. Canonne et al. (1993) Heterocycles 36:1305-1314. Little work, however, has been reported on the solid phase synthesis of fused 2,4-pyrimidinediones.

An 11 step synthesis of particularly substituted 2,4-quinazolinediones using solid phase synthesis that proceeds through an anthranilate intermediate has been described. Buckman et al. (1996) Tetrahedron Lett. 37:4439-4442. This synthetic route is inherently limited, however, to the production of phenolic 2,4-quinazolinediones due to the mode of connection between the 2,4-quinazolinedione and the solid support. Furthermore, the synthetic route is limited by the harshness of the reaction conditions employed. For instance, the cyclization of the pyrimidinedione ring requires the presence of strong

potassium hydroxide: a reagent that could cause cleavage of the compound from the solid support, destroy certain functional groups such as esters, or racemize chiral groups such as amino acid derivatives. Finally, the synthetic route requires the use of air-sensitive reagents, such as lithium benzyloxazolidinone, making the automation of the synthetic protocol difficult, thus potentially reducing its application to the manual synthesis of a limited number of 2,4-quinazolinediones.

A 5 step synthesis of 2,4-quinazolinediones using solid phase synthesis that proceeds through an anthranilate intermediate has been described. Smith et al. (1996) Biorg. & Medicinal Chem. Lett. 6:1483-1486. This route is limited in that it does not allow for the production of 2,4-quinazolinediones bound to a solid support: the last step of the synthesis both completes the pyrimidinedione ring and releases the formed compound from the solid support. The route is further limited in that it employs a moisture-sensitive chloroformate derivatized polymeric support that has to be prepared immediately before use. Finally, the final synthetic step requires a high reaction temperature virtually excluding the application of standard equipment used for the automated synthesis of combinatorial libraries. Therefore the scope of the application of this method is severely limited.

A 4 step synthesis of 2,4-quinazolinediones using solid phase synthesis that proceeds through a urethane protected anthranilamide has been described. Gouilleux et al. (1996) Tetrahedron Lett. 37:7031-7034. This route is limited in that the last step of the synthesis both completes the pyrimidinedione ring and releases the formed compound from the solid support. 2,4-quinazolinediones bound to a solid support cannot, therefore, be produced using this route.

A 4 step synthesis of pyrido[2,3-d]pyrimidines has been described. Gordeev et al. (1996) Tetrahedron Lett. 37:4643-4646. This route is limited to the production of particularly substituted pyridopyrimidines. Due to the nature of the linkage between the pyridopyrimidine and the solid support, only those compounds with a carboxyl group in the 6-position can be accessed.

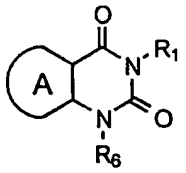
Methods for nucleophilic aromatic substitution reactions have been described, where a fluoride substituent on an aromatic ring is displaced from a chemical intermediate that is attached to a solid support. MacDonald et al. discloses the use of a nucleophilic aromatic substitution reaction on a solid support as a step in the preparation of a quinolone

library. (1996) Tetrahedron Lett. 37: 4815-4818. Phillips et al. describes the use of a nucleophilic aromatic substitution reaction in the solid phase preparation of benzimidazoles. (1996) Tetrahedron Lett. 37: 4887-4890. Shapiro et al. delineates the use of ^{19}F NMR monitoring of a nucleophilic aromatic substitution reaction on a solid support. (1996) Tetrahedron Lett. 37: 4671-4674. None of these references either disclose or suggest the use of a nucleophilic aromatic substitution reaction in the preparation of a fused 2,4-pyrimidinedione library on a solid support.

The cited references in the background section, and in the following sections, are herein incorporated by reference.

DISCLOSURE OF THE INVENTION

The present invention provides a combinatorial library that contains a fused 2,4-pyrimidinedione (shown below, I):

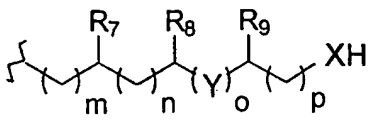


I

where the 'A' ring is an aromatic ring, a heteroaromatic ring, an aliphatic ring or substituted versions thereof.

In one embodiment, the combinatorial library contains fused 2,4-pyrimidinediones selected from the group: 2,4-quinazolinediones, pyrimidopyrimidinediones, pyridopyrimidinediones, 2,4-pteridinediones, and azolopyrimidinediones (FIG. 1, where R_2-R_6 can independently be alkyl, aryl, heteroaryl, electron withdrawing groups, and amino acid derivatives). Preferably, the combinatorial library contains fused 2,4-pyrimidinediones including but not limited to: 2,4-quinazolinediones, pyrimidopyrimidinediones, and azolopyrimidinediones.

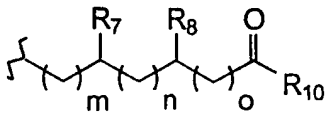
In another embodiment, the combinatorial library contains fused 2,4-pyrimidinediones, where R_1 in the fused 2,4-pyrimidinedione is:



II

where R_7, R_8, and R_9 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group alkyl, aryl, O, NH, and NR_{10}; and where XH is selected from the group CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and further where m, n, o, and p vary independently from 0 to 4, and where R_{10} and R_{11} are independently selected from the group alkyl, aryl and heteroaryl.

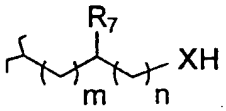
In another embodiment, the combinatorial library contains fused 2,4-pyrimidinediones, where R^1 in the fused 2,4-pyrimidinedione is:



III

where R_7 and R_8 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where m, n, and o vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from the group alkyl, aryl and heteroaryl.

In another embodiment, the combinatorial library contains fused 2,4-pyrimidinediones, where R^1 in the fused 2,4-pyrimidinedione is:



IV

where R_7 is independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, $C(O)R_{10}$, and an amino acid side chain; and where XH is selected from the group CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where m, and n vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from the group alkyl, aryl and heteroaryl.

The present invention also provides a combinatorial library that contains fused 2,4-pyrimidinediones, where the fused 2,4-pyrimidinediones are connected to a solid support via R_1, and where the fused 2,4-pyrimidinedione is selected from the group: 2,4-pteridinediones, pyridopyrimidinediones, pyrimidopyridazinediones and azolopyrimidinediones.

In one embodiment, the combinatorial library contains fused 2,4-pyrimidinediones selected from the group: 2,4-pteridinediones, pyridopyrimidinediones, and pyrimidopyridazinediones.

In another embodiment, the combinatorial library contains pyridopyrimidines.

The present invention also provides a combinatorial library that contains fused 2,4-pyrimidinediones, where the fused 2,4-pyrimidinediones are connected to a solid support through a substituent at the 3-position.

In another embodiment, the combinatorial library contains fused 2,4-pyrimidinediones selected from the group: 2,4-quinazolinediones, pyrimidopyrimidinediones, pyridopyrimidinediones, 2,4-pteridinediones, and azolopyrimidinediones.

In another embodiment, the combinatorial library contains fused 2,4-pyrimidinediones selected for the group: 2,4-quinazolinediones, pyrimidopyrimidinediones, and pyridopyrimidinediones.

In another embodiment, the combinatorial library contains a 2,4-quinazolinedione.

The present invention also provides a combinatorial library that contains fused 2,4-pyrimidinediones that is prepared by cleaving the fused 2,4-pyrimidinediones from a solid support.

The present invention also provides a combinatorial library that contains fused 2,4-pyrimidinediones that possess a substituent at the 3-position, where the library is prepared by cleaving the fused 2,4-pyrimidinedione from a solid support.

In one embodiment, this method includes a step where a fluoride substituent is displaced in a nucleophilic aromatic substitution reaction.

The present invention also provides a method of producing a combinatorial library that contains fused 2,4-pyrimidinediones, where the fused 2,4-pyrimidinediones are connected to a solid support, and where the fused 2,4-pyrimidinedione is selected from the group: 2,4-pteridinediones, pyridopyrimidinediones, pyrimidopyridazinediones and azolopyrimidinediones.

In one embodiment, this method includes a step where a fluoride substituent is displaced in a nucleophilic aromatic substitution reaction.

The present invention also provides a method of producing a combinatorial library that contains fused 2,4-pyrimidinediones, where the fused 2,4-pyrimidinediones are connected to a solid support through a substituent at the 3-position.

In one embodiment, this method includes a step where a fluoride substituent is displaced in a nucleophilic aromatic substitution reaction.

The present invention also provides a method of screening a library that contains a fused 2,4-pyrimidinedione.

In one embodiment, the fused 2,4-pyrimidinediones that are screened are connected to a solid support.

In another embodiment, the fused 2,4-pyrimidinediones that are screened are in solution.

The present invention also provides fused 2,4-pyrimidinediones that possess antimicrobial activity.

In one embodiment, the antimicrobial 2,4-pyrimidinediones contain an amino acid side chain at R_1 (Fig. 1).

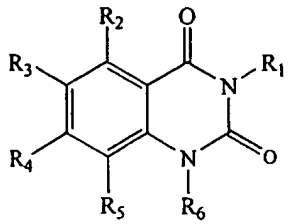
In another embodiment, the antimicrobial 2,4-pyrimidinediones contain an α-amino acid side chain at R_1 (Fig. 1).

The present invention also provides fused 2,4-pyrimidinediones that possess β-lactamase inhibitory activity.

In one embodiment, the β-lactamase inhibitory 2,4-pyrimidinediones contain an amino acid side chain at R_1 (Fig. 1).

In another embodiment, the β-lactamase inhibitory 2,4-pyrimidinediones contain an α-amino acid side chain at R_1 (Fig. 1).

The present invention also provides compounds having antimicrobial activity, said compounds having the formula



wherein R_1 is an amino acid group; R_2-R_5 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteraryl, and electron withdrawing substituents; and

R_6 is selected from the group consisting of hydrogen, alkyl, aryl, and heteroaryl substituents.

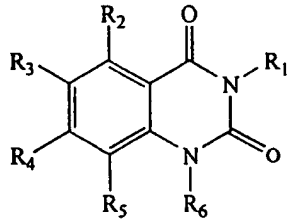
In one embodiment, R_1 is selected from the group consisting of α-amino acid groups, α-amino acid derivative groups, β-amino acid groups, and β-amino acid derivative groups.

In another embodiment, R_1 is selected from the group consisting of α-amino acid groups and α-amino acid derivative groups.

In another embodiment, R_6 is selected from the group consisting of alkyl and aryl substituents.

In another embodiment, R_6 is an alkyl substituent.

The present invention also provides compounds having β-lactamase inhibitory activity, said compounds having the formula



wherein R_1 is an amino acid group; R_2-R_5 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteraryl, and electron withdrawing substituents; and

R_6 is selected from the group consisting of hydrogen, alkyl, aryl, and heteroaryl substituents.

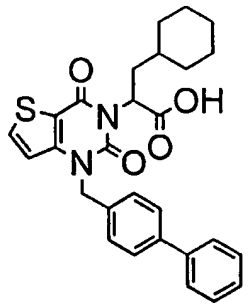
In one embodiment, R_1 is selected from the group consisting of α-amino acid groups, α-amino acid derivative groups, β-amino acid groups, and β-amino acid derivative groups.

In another embodiment, R_1 is selected from the group consisting of α-amino acid groups and α-amino acid derivative groups.

In another embodiment, R_6 is selected from the group consisting of alkyl and aryl substituents.

In another embodiment, R_6 is an alkyl substituent.

The present invention also provides bioactive thiophene-pyrimidinedione compounds (see Figure 1) having antimicrobial and/or β-lactamase inhibitory activity, such as the following thieno[2,3-d]pyrimidine-2,4-dione:



The present invention also provide pharmceutical compositions for antimicrobial use which comprise a compound of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the general chemical structures of 2,4-quinazolinediones, pyrimidopyrimidinediones, pyridopyrimidinediones, 2,4-pteridinediones, and azolopyrimidinediones.

FIG. 2 illustrates general synthetic routes to fused 2,4-pyrimidinediones using solid support technology.

FIG. 3 illustrates a synthetic route to fused 2,4-pyrimidinediones, where an immobilized amine derivative is an intermediate in the synthesis, and where the cleavage of the 2,4-pyrimidinedione from the solid support provides a terminal carboxylic acid moiety.

FIG. 4 illustrates a synthetic route to fused 2,4-pyrimidinediones, where an immobilized alkoxy amine derivative is an intermediate in the synthesis, and where the cleavage of the 2,4-pyrimidinedione from the solid support provides a terminal hydroxamic acid moiety.

FIG. 5 illustrates a synthetic route to fused 2,4-pyrimidinediones, where a 3-amino-2-carboxylate thiophene is used in place of anthranilic acid derivatives in the solid phase synthesis of fused 2,4-pyrimidinediones.

FIG. 6 illustrates a synthetic route to fused 2,4-pyrimidinediones, where 7-fluoroquinazoline-2,4-diones attached to a solid support are derivatized through nucleophilic aromatic substitution reactions.

FIG. 7 illustrates the types of reagents that can be used to derivatize 7-fluoroquinazoline-2,3-diones through nucleophilic aromatic substitution reactions.

FIG. 8 illustrates the construction and composition of a 1,200 member 2,4-quinazolinedione library.

FIG. 9 illustrates a selected group of fused 2,4-pyrimidinediones that possess antimicrobial activity and/or β-lactamase inhibitory activity.

BEST MODE FOR CARRYING OUT THE INVENTION

Definitions

The term "bioactive molecule," as used herein, refers to a molecule that inhibits the interaction between an enzyme or receptor and its respective substrate(s) or endogenous ligand(s), by at least a 15%, at a solution concentration of 10^{-3} molar or lower (i.e., it has inhibitory activity). Preferably, the molecule will inhibit such interaction at solution concentrations of 10^{-4} molar or lower. More preferably, the molecule will inhibit such interaction at solution concentrations of 10^{-5} molar or lower. Most preferably, the molecule will inhibit such interaction at solution concentrations of 10^{-6} molar or lower. The term "bioactive molecule," as used herein, also refers to a molecule that either inhibits the growth of or kills a microorganism, and causes at least a 15% reduction in the population of that microorganism, at solution concentrations 100 mg/ml or lower (i.e., it has antrimicrobial activity). Preferably, the molecule has antimicrobial activity at solution concentrations of 10 mg/ml or lower. Most preferably, the molecule antimicrobial activity at solution concentrations of 1 mg/ml or lower.

"Chemical library" or "array" is an intentionally created collection of differing molecules which can be prepared synthetically and screened for biological activity in a variety of different formats (e.g., libraries of soluble molecules, libraries of molecules bound to a solid support).

"Alkyl" refers to a cyclic, branched, or straight chain chemical group containing only carbon and hydrogen, such as methyl, pentyl, and adamantyl. Alkyl groups can either be unsubstituted or substituted with one or more substituents, e.g., halogen, alkoxy, acyloxy, amino, hydroxyl, mercapto, carboxy, benzyloxy, phenyl, benzyl, biphenyl, biphenyl ether, biphenyl benzyl ether, anthraquinone, or other functionality that may be suitably blocked, if necessary for purposes of the invention, with a protecting group. Alkyl groups can be saturated or unsaturated (e.g., containing -C=C- or -C≡C- subunits), at one or several positions. Typically, alkyl groups will comprise 1 to 12 carbon atoms, preferably 1 to 10, and more preferably 1 to 8 carbon atoms.

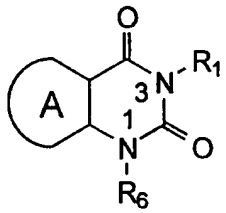
"Amino acid" refers to any of the naturally occurring amino acids, as well as optical isomers (enantiomers and diastereomers), synthetic analogs and derivatives thereof. α-Amino acids comprise a carbon atom to which is bonded an amino group, a carboxyl group, a hydrogen atom, and a distinctive group referred to as a "side chain". The side chains of naturally occurring amino acids are well known in the art and include, for example, hydrogen (e.g., as in glycine), alkyl (e.g., as in alanine, valine, leucine, isoleucine, proline), substituted alkyl (e.g., as in threonine, serine, methionine, cysteine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, and lysine), arylalkyl (e.g., as in phenylalanine and tryptophan), substituted arylalkyl (e.g., as in tyrosine), and heteroarylalkyl (e.g., as in histidine). See, e.g., Harper et al. (1977) *Review of Physiological Chemistry*, 16th Ed., Lange Medical Publications, pp. 21–24. One of skill in the art will appreciate that the term "amino acid" also includes β- γ-, δ-, and ω-amino acids, and the like. Unnatural amino acids are also known in the art, as set forth in, for example, Williams (ed.), *Synthesis of Optically Active α-Amino Acids*, Pergamon Press (1989); Evans *et al., J. Amer. Chem. Soc.*, 112:4011–4030 (1990); Pu *et al., J. Amer. Chem. Soc.*, 56:1280–1283 (1991); Williams *et al., J. Amer. Chem. Soc.*, 113:9276–9286 (1991); and all references cited therein.

"Aryl" or "Ar" refers to a monovalent unsaturated aromatic carbocyclic group having a single-ring (e.g., phenyl), multiple rings (e.g. biphenyl), or multiple condensed rings (e.g., naphthyl or anthryl), which can be optionally unsubstituted or substituted with amino, hydroxyl, lower alkyl, alkoxy, chloro, halo, mercapto, and other substituents.

"Electron withdrawing group" refers to a substituent that draws electrons to itself more than a hydrogen atom would if it occupied the same position in a molecule. Examples of electron withdrawing groups include —NR_2, —COOH, —OR, —SR_2, —F, —COR, —Cl, —SH, NO_2, —Br, —SR, —SO_2R, —I, —OH, —CN, —C=CR, —COOR, —Ar, —$CH=CR_2$, where R is akyl, aryl, arylalkyl, or heteroaryl.

"Heteroaryl" or "HetAr" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g., pryridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl) and having at least one hetero atom, such as N, O, or S, within the ring, which can optionally be unsubstituted or substituted with amino, hydroxyl, alkyl, alkoxy, halo, mercapto, and other substituents.

The "3-position" of a fused 2,4-pyrimidinedione is the nitrogen between the two carbonyl groups; the "1-position" of a fused 2,4-pyrimidinedione is the nitrogen attached to the fused ring (as shown below):

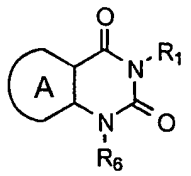


V

"Protecting group" refers to a chemical group that exhibits the following characteristics: (1) reacts selectively with the desired functionality in good yield to give a protected substrate that is stable to the projected reactions for which protection is desired; 2) is selectively removable from the protected substrate to yield the desired functionality; and 3) is removable in good yield by reagents compatible with the other functional group(s) generated in such protected reactions. Examples of protecting groups can be found in Greene et al. (1991) *Protective Groups in Organic Synthesis*, 2nd Ed. (John Wiley & Sons, Inc., New York).

Fused 2,4-Pyrimidinedione Libraries

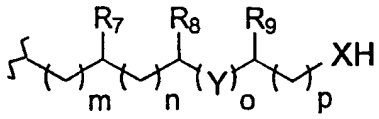
The present invention provides a combinatorial library containing 2,4-pyrimidinediones that are fused to an aliphatic, an aromatic ring, or a heteroaromatic ring. Fused 2,4-pyrimidinediones are compounds of the following general structure:



I

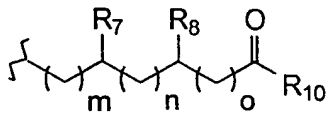
Examples of aromatic and heteroaromatic fused 2,4-pyrimidinediones include, but are not limited to, 2,4-quinazolinediones, pyrimidinediones, pyridopyrimidinediones, 2,4-pteridinediones, pyrimidopyridazinediones, and azolopyrimidinediones (shown in Fig. 1). R_2, R_3, R_4, R_5, and R_6 in Fig. 1 can independently be an alkyl group, an aryl group, a heteroaryl group, and an electron withdrawing group.

When the fused 2,4-pyrimidinedione is not attached to a solid support, R^1 can be a substituted alkyl group represented by one of the following three structures:



II

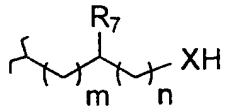
where R_7, R_8, and R_9 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group alkyl, aryl, O, NH, and NR_{10}; and where XH is selected from the group CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and further where m, n, o, and p vary independently for 0 to 4; and where R^{10} and R^{11} are independently selected from alkyl, aryl, and heteroaryl; or



III

where R_7 and R_8 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where m, n,

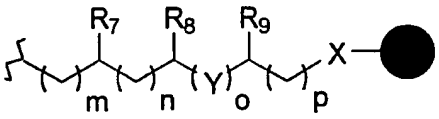
and o vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from alkyl, aryl, and heteroaryl ; or,



IV

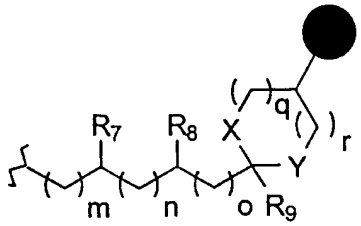
where R_7 is independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where XH is selected from the group CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where m and n vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from alkyl, aryl, and heteroaryl.

When the 2,4-pyrimidinedione library is attached to a solid support, R_1 can be a substituted alkyl group represented by one of the following three structures, where the solid support is represented by a darkened circle:



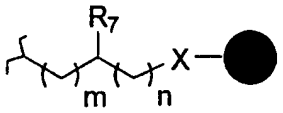
V

where R_7, R_8, and R_9 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where Y is selected from the group alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and further where m, n, o, and p vary independently for 0 to 4; and where R_{10} and R_{11} are independently selected from alkyl, aryl, and heteroaryl; or,



VI

where R_7, R_8, and R_9 are independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where X and Y are independently selected from the group O and S; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from alkyl, aryl, and heteroaryl; or,



VII

where R_7 is independently selected from the group H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and a natural amino acid side chain; and where X is selected from the group CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from alkyl, aryl, and heteroaryl.

Bioactive 2,4-Pyrimidinediones

The present invention provides bioactive, fused 2,4-pyrimidinediones, where the R_1 substituent (FIG. 1) is an amino acid. These bioactive compounds possess β-lactamase activity and/or antimicrobial activity. A group of selected, fused 2,4-pyrimidinediones possessing such activity is shown in Figure 9.

Compounds that possess antimicrobial activity may be used *in vitro*, or may be administered to treat either humans or animals that are infected with a corresponding pathogenic microbe. Compounds that possess β-lactamase activity may be used *in vitro*, or may be administered with a β-lactam antibiotic, therefore increasing the *in vivo* half-life of the antibiotic, to treat infectious diseases that affect either humans or animals. Combination therapies are very common and often effective in anti-infective treatments. In this way, one or more bioactive compounds of the present invention may be used in conjunction with other bioactive compounds outside the present invention in a combination therapy.

Overview of Combinatorial Synthesis

Combinatorial library synthesis is typically performed on a solid support. See, for example, Lam et al. (1991) Nature 354:82-84; Houghten et al. (1991) Nature 354:84-86. A large number of beads or particles are suspended in a suitable carrier (such as a solvent) in a parent container. The beads, for example, are provided with a functionalized point of attachment for a chemical module. The beads are then divided and placed in various separate reaction vessels. The first chemical module is attached to the bead, providing a variety of differently substituted solid supports. Where the first chemical module includes 3 different members, the resulting substituted beads can be represented as A_1, A_2, and A_3.

The beads are washed to remove excess reagents and subsequently remixed in the parent container. This bead mixture is again divided and placed into various separate reaction vessels. The second chemical module is coupled to the first chemical module. Where the second chemical module includes 3 different members, B_1, B_2, and B_3, 9 differently substituted beads result: A_1B_1, A_1B_2, A_1B_3, A_2B_1, A_2B_2, A_2B_3, A_3B_1, A_3B_2, and A_3B_3. Each bead will have only a single type of molecule attached to its surface.

The remixing/redivision synthetic process can be repeated until each of the different chemical modules has been incorporated into the molecule attached to the solid support. Through this method, large numbers of individual compounds can be rapidly and efficiently synthesized. For instance, where there are 4 different chemical modules, and where each chemical module contains 20 members, 160,000 beads of different molecular substitution can be produced.

Combinatorial library synthesis can be performed either manually or through the use of an automated process. For the manual construction of a combinatorial library, a scientist would perform the various chemical manipulations. For the construction of a combinatorial library through an automated process, the various chemical manipulations will typically be performed robotically. For example, see U.S. Patent No. 5,463,564.

Solid Supports

The synthesis of a 2,4-pyrimidinedione library can be performed on a solid support. "Solid support" includes an insoluble substrate that has been appropriately derivatized such that a chemical module can be attached to the surface of the substrate through standard

chemical methods. Solid supports include, but are not limited to, beads and particles, such as peptide synthesis resins. For example, see Merrifield (1963) J. Am. Chem. Soc. 85:2149-2154; U.S. Patent No. 4,631,211; and Geysen et al. (1984) Proc. Natl. Acad. Sci. USA 81:3998-4002.

Solid supports can consist of many materials, limited primarily by the capacity of the material to be functionalized through synthetic methods. Examples of such materials include, but are not limited to, polymers, plastics, resins, polysaccharides, silicon or silica based materials, carbon, metals, inorganic glasses and membranes. Preferred resins include Sasrin resin (a polystyrene resin available from Bachem Bioscience, Switzerland), and TentaGel S AC, TentaGel PHB, or TentaGel S NH_2 resin (polystyrene-polyethylene glycol copolymer resins available from Rapp Polymere, Tubingen, Germany).

The solid support can be purchased with suitable functionality already present such that a chemical module can be attached to the support surface (e.g., Novabiochem, Bachem Bioscience, Rapp Polymere). Alternatively, the solid support can be chemically modified such that a chemical module can be attached to the support surface. Grant (1992) *Synthetic Peptides. A User's Guide*, W.H. Freeman and Co; Hermkens et al. (1996) Tetrahedron 52:4527-4554. The choice of functionality used for attaching a molecule to the solid support will depend on the nature of the compound to be synthesized and the type of solid support. Examples of functionality present on the solid support that can be used to attach a chemical module, include, but are not limited to, alkyl or aryl halides, aldehydes, alcohols, ketones, amines, sulfides, carboxyl groups, aldehyde groups, and sulfonyl groups.

Preferably, the functional group on the solid support that permits the attachment of a chemical module will be an alcohol, an amine, an aldehyde, or a diol group. Gordon et al. (1994) J. Med. Chem. 37:1385-1401; Hermkens et al. (1996) Tetrahedron 52:4527-4554.

For certain combinatorial libraries, one can purchase a solid support with an existing, protected chemical module already attached. An example of such a support is FmocGly Sasrin, which is commercially available from Rapp Polymere. Typically, however, the first step of the combinatorial library synthesis is the attachment of a chemical module to the solid support through the existing functionality on the support surface. Examples of chemical reactions that can be used to attach a chemical module to the support include, but are not limited to, nucleophilic displacement of a halide or other

leaving group, etherification of an alcohol, esterification of an alcohol, amidation of an amine, acetalization of an aldehyde, and ketalization of a ketone. Hermkens et al. (1996) Tetrahedron 52:4527-4554.

Preferably, the reaction used to attach the chemical module to the solid support will be an esterification of an alcohol, an amidation of an amine, or an acetalization of an aldehyde. For example, see Hermkens et al. (1996) Tetrahedron 52:4527-4554.

For the attachment of certain chemical modules to the solid support, masking of functionality that is not involved in the attachment process, but that is incompatible with the mode of attachment, may be necessary. A non-limiting example of this type of process is the esterification of an alcohol functionalized solid support, using a hydroxyl-substituted carboxylic acid as the coupling partner. Prior to the esterification reaction, the hydroxyl group of the carboxylic acid would be "protected" through alkylation, silylation, acetylation, or through some other standard method. Strategies for the use of masking or protecting groups have been well-described in the art, such as in Green (1985) *Protecting Groups in Organic Synthesis*, Wiley.

Nucleophilic Aromatic Substitution Reactions

Nucleophilic substitution reactions at aromatic carbon centers typically proceed too slowly to be of synthetic utility. Under 4 different scenarios, however, there are exceptions to this rule: 1) where an electron withdrawing group is either *ortho* or *para* to the leaving group; 2) where a strong base forms an aryne intermediate that is subject to nucleophilic attack; 3) where the nucleophile can donate an electron through a transfer mechanism; 4) where a diazonium salt is replaced. see March, "Advanced Organic Chemistry," John Wiley & Sons, New York, 1985. Of these 4 mechanisms, the first is the most utilized form.

The displacement of a fluoride substituent from an aromatic ring that contains a *para* carbonyl group is a version of the first nucleophilic aromatic substitution reaction mechanism. The carbonyl group can be an ester, ketone, heterocyclic vinylogous amide, or heterocyclic conjugated ketone. Luo et al. (1994) J. Org. Chem. 1761-1765; Berge et al. (1994) Synlett 187-188; Inoue et al. (1994) J. Med. Chem. 586-592; Cecchetti et al. (1993) J. Het. Chem. 1143-1148. Although 1 carbonyl group is sufficient to promote nucleophilic

aromatic substitution, this reaction can be facilitated by the addition of a second or third electron withdrawing group to the aromatic ring. Examples of such electron withdrawing groups are shown in Figure 7.

A variety of nucleophiles can be used to displace fluoride from an aromatic ring containing a *para* carbonyl group. Suitable nucleophilic reagents include, but are not limited to, amines, hydrazines, hydroxylamines, NH-heterocycles, alcohols, and thiols. Inoue et al. (1994) J. Med. Chem. 586-592; Cecchetti et al. (1993) J. Het. Chem. 1143-1148; Kalindjian et al. (1991) Synlett 803-804; Ziegler et al. (1988) J. Het. Chem. 1543; Cooper et al. (1992) J. Med. Chem. 1392-1398; Luo et al. (1994) J. Org. Chem. 1761-1765; Stabler et al. (1994) Synth. Commun. 123-129; Yeager et al. (1991) Synthesis 63-68; Berge et al. (1994) Syntlett 187-188; Guggenheim (1987) Tetrahedron Lett. 6139; Gornostaev et al. (1992) Zh. Org. Khim. 2291-2293.

Synthetic Routes to Fused 2,4-Pyrimidinedione Libraries

A general synthetic strategy for the construction of fused 2,4-pyrimidinedione containing libraries, delineating two possible synthetic routes, is shown in Figure 2. The first route employs the addition of an immobilized amine derivative to an isocyanate or to an activated carbamate. The second route employs the addition of an aniline derivative to an immobilized isocyanate or to an immobilized activated carbamate.

To construct a 2,4-pyrimidinedione library through the immobilized amine derivative route, a chemical module containing a terminal amine, or protected terminal amine, is attached to a functionalized resin. Where the terminal amine of the chemical module is protected, the synthetic route proceeds through the deprotection of the terminal amine. An isocyanate or activated carbamate derived from an anthranilate or heterocyclic anthranilate is added to the immobilized amine to form a urea derivative. The urea derivative is treated with a base, producing a fused 2,4-pyrimidinedione attached to the solid support. Substitution of N^1 can be effected upon treatment of the immobilized, fused 2,4-pyrimidinedione with a base and an alkylating agent. Among other methods, such N^1-alkylation can also be achieved upon treatment of the immobilized fused 2,4-pyrimidinedione with an alcohol in the presence of a phosphine derivative and an alkyl

azodicarboxylate. The fused 2,4-pyrimidinedione can be cleaved from the solid support, providing a fused 2,4-pyrimidinedione in solution.

Figure 3 shows a specific embodiment of the immobilized amine route, where the synthesized, fused 2,4-pyrimidinedione is a 2,4-quinazolinedione with an amino acid derived substituent at the 3-position. An Fmoc protected, amino acid modified, Sasrin resin is treated with piperidine to produce the unprotected, bound amino acid. To the bound amino acid is added 2-carboxymethyl phenylisocyanate. The resulting urea is cyclized upon treatment with DBU to form a solid support bound 2,4-quinazolinedione. This compound is alkylated upon the addition of an alkylating agent in the presence of a base. Cleavage of the synthesized compound is effected by treatment of the bound 2,4-quinazolinedione with triflouroacetic acid. Figure 4 shows a slightly modified synthetic route for the construction of hydroxamic acids.

To construct a fused 2,4-pyrimidinedione library through the immobilized isocyanate or immobilized activated carbamate route, a chemical module containing a terminal amine, or protected terminal amine, is attached to a functionalized resin. Where the terminal amine of the chemical module is protected, the synthetic route proceeds through the deprotection of the terminal amine. The deprotected terminal amine is converted to either an isocyanate or to an activated carbamate. An anthranilate, anthranilic acid, heterocylic anthranilate or heterocyclic anthranilic acid is added to the isocyanate or activated carbamate, forming a urea derivative. The urea derivative is treated with a base, producing a fused 2,4-pyrimidinedione attached to the solid support. Substitution of N^1 can be effected upon treatment of the immobilized, fused 2,4-pyrimidinedione with a base and an alkylating agent. The fused 2,4-pyrimidinedione can be cleaved from the solid support, providing a 2,4-pyrimidinedione in solution.

Figure 5 shows a specific embodiment of the immobilized isocyanate or immobilized activated carbamate route, where the synthesized, fused 2,4-pyrimidinedione is a 2,4-quinazolinedione with an amino acid derived substituent at the 3-position. An amino acid modified Sasrin resin is treated with either triphosgene and lutidine to produce the isocyanate, or with 4-nitrophenyl chloroformate and lutidine to produce the activated carbamate. Addition of methyl anthranilate to the isocyanate or the activated carbamate yields the corresponding urea. This urea is cyclized upon heating and treatment with

DBU. The resulting bound 2,4-quinazolinedione is then cleaved by the addition of dilute trifluoroacetic acid.

Figure 6 shows a specific embodiment of the immobilized amine route, where the substituent at the 7-position of the 2,4-quinazolinedione derivatives can be altered through the nucleophilic displacement of fluoride. An anthranilic acid derivative containing a fluoride substituent group *para* to the carboxylic acid is treated with triphosgene followed by potassium hydroxide in methanol to produce the corresponding methyl ester. The aniline group is converted to an activated carbamate upon treatment with 4-nitrophenyl chloroformate. Attachment of this intermediate is effected by reaction with H-Phe-Sasrin, which links the compound to the resin through the resultant urea functionality. The urea is cyclized upon treatment with base to form a 2,4-quinazolinedione derivative. Displacement of the fluoride substituent through nucleophilic displacement with an amine provides the desired solid support bound 2,4-quinazolinedione analogue. This analogue can be cleaved from the sasrin resin through treatment with trifluoroacetic acid.

A solid support bound, fused 2,4-pyridopyrimidine library can be recovered through conventional methods such as filtration or centrifugation. Confirmation that the solid support contains the desired fused 2,4-pyridopyrimidine compound can be accomplished by cleaving the fused 2,4 pyridopyrimidine from a small portion of the solid support, and then subjecting the cleaved product to conventional analysis. Examples of commonly used analytical methods include, but are not limited to, nuclear magnetic resonance spectroscopy and high performance liquid chromatography.

Methods of Cleavage

In one embodiment of the invention, the fused 2,4-pyrimidinedione library is bound to a solid support. In another embodiment of the invention, the fused 2,4-pyrimidinedione is cleaved from the solid support to produce soluble fused 2,4-pyrimidinedione libraries. Soluble libraries can be advantageous for a variety of purposes, including assaying the biological activity of compounds and performing structural analysis of compounds.

The cleavage of compounds from a solid support to produce a soluble chemical library can be accomplished using a variety of methods. For example, a compound can be photolytically cleaved from a solid support (Wang et al. (1976) J. Org. Chem. 41:3258;

Rich et al. (1975) J. Am. Chem. Soc. 97:1575-1579), and through nucleophilic attack (U.S. Patent No. 5,549,974), or through hydrolysis (Hutchins et al. (1994) Tetrahedron Lett. 35:4055-4058).

Preferably, the cleavage of compounds from a solid support to produce a soluble chemical library is accomplished using hydrolytic conditions, such as through the addition of dilute trifluoroacetic acid.

Screening

The present invention is directed toward the generation of fused 2,4-pyrimidinedione libraries. These libraries may be used to select one or more fused 2,4-pyrimidinedione species that possess activity against a cellular target, including but not limited to enzymes and receptors, or a microorganism. A cellular ligand or microorganism is targeted when it is believed that it is of importance in the etiology or progression of a disease. Examples of disease states for which fused 2,4-pyrimidinedione libraries can be screened include, but are not limited to, inflammation, infection, hypertension, CNS disorders, and cardiovascular disorders.

Several methods have been developed in recent years to screen libraries of compounds to identify the compounds having the desired characteristics. Typically, where a compound exhibits a dissociation constant of 10^{-6} or less when combined with the targeted enzyme or receptor, the compound is thought to demonstrate a specific interaction with the enzyme or receptor. Methods for isolating library compound species that demonstrate desirable affinity for a receptor or enzyme are well-known in the art.

For example, an enzyme solution may be mixed with a solution of the compounds of a particular combinatorial library under conditions favorable to enzyme-ligand binding. See Bush et al. (1993) Antimicrobial Agents and Chemotherapy 37:851-858, and Daub et al. (1989) Biochemistry 27:3701-3708. Specific binding of library compounds to the enzyme may be detected by any of the numerous enzyme inhibition assays which are well known in the art. Compounds which are bound to the enzyme may be readily separated from compounds which remain free in solution by applying the solution to a Sephadex G-25 gel filtration column. Free enzyme and enzyme-ligand complexes will pass through the column quickly, while free library compounds will be retarded in their progress through

the column. The mixture of enzyme-ligand complex and free enzyme can then be treated with a powerful denaturing agent, such as guanidinium hydrochloride or urea, to cause release of the ligand from the enzyme. The solution can then be injected onto an HPLC column (for example, a Vydac C-4 reverse-phase column, eluted with a gradient of water and acetonitrile ranging from 0% acetonitrile to 80% acetonitrile). Diode array detection can provide discrimination of the compounds of the combinatorial library from the enzyme. The compound peaks can then collected and subjected to mass spectrometry for identification.

An alternate manner of identifying compounds that inhibit an enzyme is to divide the library into separate sublibraries where one step in the synthesis is unique to each sublibrary. To generate a combinatorial library, reactants are mixed together during a step to generate a wide mixture of compounds. At a certain step in the synthesis, however, the resin bearing the synthetic intermediates can be divided into several portions, with each portion then undergoing a unique transformation. The resin portions are then (separately) subjected to the rest of the synthetic steps in the combinatorial synthetic method. Each individual resin portion thus constitutes a separate sublibrary. When testing the compounds, if a given sublibrary shows more activity than the other sublibraries, the unique step of that sublibrary may then be held fixed. The sublibrary then becomes the new library, with that step fixed, and forms the basis for another round of sublibrary synthesis, where a different step in the synthesis is optimized. This procedure can be executed at each step until a final compound is arrived at. The aforementioned method is the generalization of the method described in Geysen, WO 86/00991, for determining peptide "mimotopes," to the synthetic method of this invention.

Finding a compound that inhibits an enzyme is most readily performed with free compound in solution. The compounds can also be screened while still bound to the resin used for synthesis; in some applications, this may be the preferable mode of finding compounds with the desired characteristics. For example, if a compound that binds to a specific antibody is desired, the resin-bound library of compounds may be contacted with an antibody solution under conditions favoring a stable antibody-compound-resin complex. A fluorescently labeled second antibody that binds to the constant region of the first antibody may then be contacted with the antibody-compound-resin complex. This will allow identification of a specific bead as carrying the compound recognized by the first

antibody binding site. The bead can then be physically removed from the resin mixture and subjected to mass spectral analysis. If the synthesis has been conducted in a manner such that only one compound is likely to be synthesized on a particular bead, then the binding compound has been identified. If the synthesis has been carried out so that many compounds are present on a single bead, the information derived from analysis can be utilized to narrow the synthetic choices for the next round of synthesis and identification.

The enzyme, antibody, or receptor target need not be in solution either. Antibody or enzyme may be immobilized on a column. The library of compounds may then be passed over the column, resulting in the retention of strongly binding compounds on the column after weaker-binding and non-binding compounds are washed away. The column can then be washed under conditions that dissociate protein-ligand binding, which will remove the compounds retained in the initial step. These compounds can then be analyzed, and synthesized separately in quantity for further testing. Similarly, cells bearing surface receptors can be expressed on a cell surface may be contacted with a solution of library compounds. The cells bearing bound compounds can be readily separated from the solution containing non-binding compounds. The cells can then be washed with a solution which will dissociate the bound ligand from the cell surface receptor. Again, the cells can be separated from the solution, and the solution analyzed.

Pharmaceutical Compositions

The present invention also provides a pharmaceutical composition which comprises a bioactive fused 2,4-pyrimidinedione compound or a pharmaceutically acceptable salt or in vivo hydrolyzable ester thereof and a pharmaceutically acceptable carrier. The compositions of the invention include those in a form adapted for oral, topical or parenteral use and may be used for the treatment of bacterial infection in mammals including humans.

The antibiotic compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics.

The composition may be formulated for administration by any route, such as oral, topical or parenteral. The compositions may be in the form of tablets, capsules, powders,

granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods will known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavoring or coloring agents.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously,

agents such as a local anesthetic preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will preferably contain from 50-500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

The following examples are provided to illustrate, but not limit, the invention.

EXAMPLES

General Methods

Reagents were purchased from Aldrich, Sigma, Bachem Biosciences and Rapp Polymere and used without further purification. Immobilized N-Fmoc-protected amino acids were prepared from commercial Fmoc-amino acids using standard coupling protocols, (Grant (1992) *Synthetic Peptides. A User's Guide.* W.H. Freeman and Co.) or purchased from Bachem Bioscience.

After workup, concentration of solutions was performed by reduced pressure rotary evaporation, or using the Savant's SpeedVac instrument.

NMR spectra were recorded on a Varian Gemini 300 Mhz instrument with $CDCl_3$ as solvent unless noted. ^1H NMR data are reported as follows: chemical shifts relative to

tetramethylsilane (0.00 ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet), coupling, and integration. Assignment of protons was aided by decoupling experiments. Mass-spectra were obtained using ESI technique. HPLC analysis and purification were performed using Beckman System Gold^R; detection at 220 nm. Analytical HPLC was performed on Rainin Microsorb C18 (4.6 mm x 150 mm) reverse phase column (gradient from 100% of the aq. 0.1% TFA to 100% of 0.1% TFA in MeCN over 35 min, flow rate 1.0 mL/min). For the 7-substituted 2,4-quinazolinediones, analytical HPLC was performed on YMC 4.6 x 500 mm reverse phase column using a gradient from 90% of the aq. 0.1% TFA (eluent A)--10% of 0.1% TFA in MeCN (eluent B) to 100% of the eluent B over 6 min., flow rate 2.0 mL/min.

General Procedures for Solid Phase Preparations of N^1-Unsubstituted Fused 2,4-Pyrimidinediones

Method A. From Immobilized Amine Reagents with Isocyanates or Activated Carbamates in Solution. An appropriate N-Fmoc-protected amino acid resin [0.06 mmol, ca. 100 mg for the Sasrin support immobilized amines] was deprotected with 20% piperidine in dimethylformamide for 30 min. The resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. The amine resin was suspended with an appropriate isocyanate or p-nitrophenylcarbamate (0.2-0.5 mmol) in 10% pyridine in dimethylformamide (1-2 mL), and agitated at room temperature (r.t.). until a negative ninhydrine test indicated the absence of a free amine on a solid phase (typically, 0.5-3 h for reactions with isocyanates, or 1-24 h for reactions with p-nitrophenylcarbamate). The resultant urea resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. Immobilized urea derivatives thus obtained were further cyclized into fused 2,4-pyrimidinediones by agitation at 40-80 °C (preferably at 50-65 °C) with an organic (such as 2-10% 1,8-diaza-bycyclo[5.4.0]undec-7-ene, 1,4-diazabycyclo[2.2.2]octane, 1,5-diazabycyclo[4.3.0]-non-5-ene, or tetramethylguanidine in dimethylformamide, N-methylpyrrolidine-2-one, and like polar solvents) or inorganic (such as 1-10% lithium, sodium, or cesium carbonates in dimethylformamide or N-methylpyrrolidine-2-one) bases for 2-24 h. The resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum.

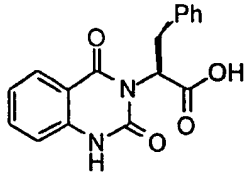
The resultant fused 2,4-pyrimidinediones were cleaved from supports with 1-40% TFA in CH_2Cl_2 for 0.5-2 h. Thus, the Sasrin resin immobilized products were typically released from support with 1% TFA in CH_2Cl_2 (30 min). When necessary, amino acid side chain functionalities were further deprotected with mixtures of TFA and additives (scavengers: thiols, phenols, or trialkylsilanes), such as 5% triethylsilane - 40% TFA in CH_2Cl_2 (0.5-4 h, depending on the nature of the protection groups). The crude products were lyophilized and analyzed by NMR, MS, and HPLC.

Method B. From Immobilized Isocyanates with Amine Reagents in Solution. An appropriate amine resin (such as immobilized amino acid reagents, see above, Method A; 0.06 mmol, ca. 100 mg for Sasrin support) was agitated with triphosgene (60 mg, 0.19 mmol) and organic base (such as 2,6-lutidine, 0.3 ml) in CH_2Cl_2 (1.5 ml) for 0.5-1.5 h (until a negative ninhydrin test indicated the absence of a free amine on a solid phase). The resultant isocyanate resin was washed liberally with CH_2Cl_2, and an appropriate amine (such as methyl anthranilate, 1 mmol) with 2,6-lutidine (0.2 ml) in CH_2Cl_2 (2 ml) was added. The mixture was agitated at r.t. until the reaction was completed (typically, 2-8 h). The resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. The resultant immobilized ureas were further converted into fused 2,4-pyrimidinediones analogously to the Method A.

Method C. From Immobilized Activated Carbamates with Amine Reagents in Solution. An appropriate amine resin (such as immobilized amino acid reagents, see above, Method A; 0.06 mmol, ca. 100 mg for Sasrin support) was agitated with p-nitrophenyl chloroformate (202 mg, 1.0 mmol) and organic base (such as 2,6-lutidine, 0.3 ml) in CH_2Cl_2 (1.5 ml) for 1-2 h (until a negative ninhydrine test indicated the absence of a free amine on a solid phase). The resultant p-nitrophenylcarbamate resin was filtered, washed liberally with CH_2Cl_2, dried under vacuum (r.t., 0.5 Torr). An appropriate amine (such as methyl anthranilate, 1 mmol) and a solution of organic base such as 10% pyridine or 2,6-lutidine in dimethylformamide (2 ml) was added, and the mixture agitated at 20-70 °C for 8-24 h (typically, this reaction with methyl anthranilates was essentially completed overnight at r.t.). The resin was filtered, washed liberally with dimethylformamide,

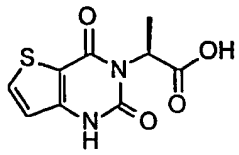
MeOH, and CH_2Cl_2, and dried under vacuum. The resultant immobilized ureas were further converted into fused 2,4-pyrimidinediones analogously to the Method A.

3-[(S)-1-Benzyl-1-carboxymethyl]-2,4-(1H, 3H)-quinazolinedione.



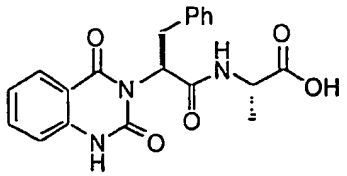
The compound was prepared from the Fmoc-Phe-Sasrin resin with 2-methoxycarbonylphenylisocyanate (Method A), or with triphosgene and methyl anthranilate (Method B), or with p-nitrophenyl chloroformate and methyl anthranilate (Method C) of the General Procedures for Solid Phase Preparations of Fused 2,4-Pyrimidinediones. HPLC purity 94%. R_t 14.0. ^{1}H NMR in $CDCl_3$ (δ, ppm): 3.35 (m, 2 H), 5.68 (m, 1 H), 6.84 (d, J = 8.7 Hz, 1 H), 6.90-7.02 (m, 6 H), 7.38 (m, 1 H), 7.83 (d, J = 8.7 Hz, 1 H), 9.97 (br. s, 1 H). Mass-spectrum (m/z): 311 $(M+H)^+$.

3-[(S)-1-Carboxyethyl]-2,4-(1H, 3H)-thieno[2,3-d]pyrimidinedione.



The compound was prepared from the FmocAla-Sasrin resin with 3-(p-nitrophenyl)carbamoyl-2-methoxycarbonylthiophene (Method A) of the General Procedures for Solid Phase Preparations of Fused 2,4-Pyrimidinediones. HPLC purity 94%. R_t 15.6 min. ^{1}H NMR in CD_3OD (δ, ppm): 1.57 (d, J = 6.9 Hz, 3 H), 5.53 (m, 1 H), 6.94 (d, J = 5.1 Hz, 1 H), 7.95 (d, J = 5.1 Hz, 1 H).

3-[(S)-1-Benzyl-1-[(S)-2-carbonylaminopropionic acid]methyl-2,4-(1H, 3H)-quinazolinedione.



Commercial Fmoc-Ala-Sasrin resin (0.06 g, ca. 0.036 mmol) was deprotected by agitation with 20% piperidine in dimethylformamide (1 ml, 30 min at r.t.), filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. Fmoc-Phe-OH (0.172 g, 0.445 mmol), 1-hydroxybenzotriazole (0.068 g, 0.445 mmol), and diisopropylcarbodiimide (0.07 mL, 0.445 mmol) were mixed in N-methylpyrrolidine-2-one (1 ml), and the mixture stirred at r.t. for 20 min. The resultant solution was added to the above deprotected H-Ala-Sasrin, and the mixture agitated by gentle shaking for 1.5 h. The FmocPhe-Ala-Sasrin dipeptide resin thus obtained was deprotected by agitation with 20% piperidine in dimethylformamide (1 ml, 30 min at r.t.), filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. 2-Methoxycarbonylphenylisocyanate (0.089 g, 0.5 mmol) in 10% pyridine in dimethylformamide (1 ml) was added to the deprotected dipeptide amine resin, and the mixture agitated for 1 h. The resultant urea resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, dried under vacuum, and then cyclized by stirring at 60 °C with 5% tetramethylguanidine or 5% 1,8-diaza-bycyclo[5.4.0]undec-7-ene in N-methylpyrrolidine-2-one (1 ml) for 21 h. The resultant quinazolinedione resin was cleaved with 3% trilfluoroacetic acid in CH_2Cl_2, and the product was isolated and analyzed as described above (see the General Procedures for Solid Phase Preparations of N^1-Unsubstituted Fused 2,4-Pyrimidinediones). HPLC purity 90%. R_t 16.3. ^1H NMR in CD_3OD (δ, ppm): 1.33 (d, J = 7.5 Hz, 3 H), 3.40-3.60 (m, 2 H), 4.48 (m, 1 H), 5.82 (m, 1 H), 7.00-7.20 (m, 7 H), 7.58 (m, 1 H), 7.90 (dd, J = 7.8 and 1.2 Hz, 1 H).

Table 1. Other Cleaved N^1-H Quinazolinediones made using the immobilized amine method, where R_1 is an amino acid derivative.

Table 1. Cleaved N^1- H Quinozalinediones **5***

Entry #	Amino Acid	HPLC Purity for Products **5**, %
1	Ala	95
2	Val	90
3	Ile	81
4	Met	92
5	Phe	94
6	Tyr(^{t}Bu)	97
7	Asp(^{t}Bu)	92
8	Glu(^{t}Bu)	93
9	Arg(Mtr)	95
10	Lys(Boc)	85
11	Trp(Boc)	92

*Cleaved with 1 % TFA in DCM. No N^1-alkylation was performed
Structures were in agreement with NMR and MS data.

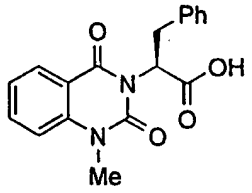
General Procedures for Solid Phase Preparations of N^1-Alkylated Fused 2,4-Pyrimidinediones

Method A. From Alkyl Halides. An appropriate N^1-H quinazolinedione resin was prepared as discussed above (0.06 mmol, ca. 100 mg for Sasrin support) was agitated with appropriate alkylating reagents (1.2 mmol) and organic base (such as tetramethylguanidine, 1,8-diazaby-cyclo[5.4.0]undec-7-ene and alike, 1.2 mmol) in N-methylpyrrolidine-2-one (1.75 ml) for 10-48 h at 20-70 °C(typically, 18 h at r.t. for examples given in the Table 2). The resultant resin was filtered, washed liberally with CH_2Cl_2, MeOH, and dried under vacuum (r.t., 0.5 Torr). Cleavage and isolation of the N^1-alkylated quinazolinediones was performed as described above for preparations of N^1-H quinazolinediones (see Method A).

Method B. From Alcohols. An appropriate N^1-H quinazolinedione resin was prepared as discussed above in *General Procedures for Solid Phase Preparations of N^1-Unsubstituted Fused 2,4-Pyrimidinediones* (0.06 mmol, ca. 100 mg for Sasrin support) with an appropriate alcohol (2.4 mmol), trisubstituted phosphine (such as triphenylphosphine, 0.472 g, 1.8 mmol), and dialkyl azodicarboxylate (such as diisopropyl azodicarboxylate, 0.283 mL, 1.8 mL) in aprotic organic solvent (such as 1,4-dioxane, 3.6 mL) was agitated at r.t. for 4-24 h (typically overnight). The resultant resin was filtered,

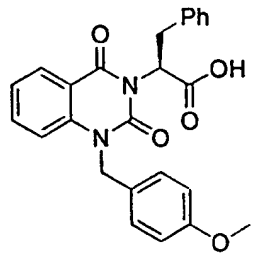
washed liberally with CH_2Cl_2, MeOH, and dried under vacuum (r.t., 0.5 Torr). Cleavage and isolation of the N^1-alkylated quinazolinediones was performed as described above for preparations of N^1-H quinazolinediones (see above, Method A).

3-[(S)-1-Benzyl-1-carboxymethyl]-1-methyl-2,4-(1H, 3H)-quinazolinedione.



The compound was prepared from the Fmoc-Phe-Sasrin resin via alkylation of the Sasrin support immobilized 3-[(S)-1-benzyl-1-carboxymethyl]-2,4-(1H, 3H)-quinazolinedione (see the example described above) according to Method A and Method B of the *General Procedures for Solid Phase Preparations of N^1-Alkylated Fused 2,4-Pyrimidinediones*. ^{1}H NMR in CD_3OD (δ, ppm): 3.25-3.58 (m, 2H), 3.51 (s, 3H), 5.89 (m, 1H), 7.00-7.20 (m, 5H), 7.26 (m, 1H), 7.37 (d, J = 9.0 Hz, 1H), 7.73 (dd, J = 7.2, 8.4 Hz, 1H), 8.02 (d, J = 7.5 Hz, 1H). Mass-spectrum (m/z): 325 $(M+H)^+$.

3-[(S)-1-Benzyl-1-carboxymethyl]-1-(p-methoxy)benzyl-2,4-(1H, 3H)-quinazolinedione.



The compound was prepared from the Fmoc-Phe-Sasrin resin via alkylation of the Sasrin support immobilized 3-[(S)-1-benzyl-1-carboxymethyl]-2,4-(1H, 3H)-quinazolinedione (see the example described above) according to Method A and Method B of the *General Procedures for Solid Phase Preparations of N^1-Alkylated Fused 2,4-Pyrimidinediones*. ^{1}H NMR in CD_3OD (δ, ppm): 3.56 (m, 2H), 4.89 (s, 3H), 5.08 (d, J =

16.2 Hz, 1H), 5.37 (d, J = 16.2 Hz, 1H), 6.00 (m, 1H), 6.82 (d, J = 8.4 Hz, 2H), 6.90-7.23 (m, 7H), 7.56 (dd, J = 8.4 and 7.2 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H). Mass spectrum (m/z): 431 $(M+H)^+$.

Table 2. Other alkylated quinazolinediones made according to Method A and Method B.

Table 2. Quinozalinediones **6** made from Phe

Entry #	R_2X	HPLC Purity for Products **6***, %
1	MeI	88
2	$BrCH_2CH_2OCH_2CH_2OMe$	75
3	$ClCH_2Ph$	94
4	$ICH_2(CH_2)_4Me$	99
5	2-$BrCH_2$-Naphthalene	99
6	N-($BrCH_2CH_2CH_2$)-Phthalimide	97
7	$BrCH_2C_6H_4OMe$-p	94
8	$BrCH_2C_6H_4NC$-o	91
9	$BrCH_2CO_2{}^tBu$	93
10	$BrCH_2CONH_2$	93

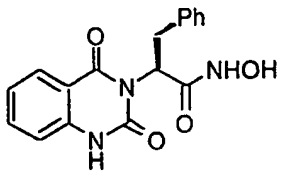
*Cleaved with 1 % TFA in DCM.

General Procedure for Solid Phase Preparations of the Fused 2,4-Pyrimidinedione Hydroxamate Derivatives

Diisopropyl azodicarboxylate (1.75 ml, 8.90 mmol) was added under inert atmosphere at ca. 10 °C to the mixture of an appropriate alcohol resin (such as Sasrin resin, 1.0 g, 0.89 mmol) with N-hydroxyphthalimide (1.45 g, 0.89 mmol) and triphenylphosphine (2.33 g, 8.90 mmol) in 1,4-dioxane (30 ml), and the mixture agitated for 24 h. Alternatively, haloalkyl-functionalized resin (such as chlorotrityl resin, 1.00 g, 0.67 mmol) was agitated with N-hydroxyphthalimide 1.10 g, 6.8 mmol) and diisopropylethylamine (2.36 ml, 13.6 mmol) in 1,4-dioxane (20 ml) and CH_2Cl_2 (4 ml) for ca. 17 h. The resin was filtered, washed liberally with CH_2Cl_2, MeOH, and dried under vacuum (r.t., 0.5 Torr). The resulted O-immobilized N-hydroxyphthalimide resins were agitated with methylhydrazine (0.47 ml, 8.9 mmol) in CH_2Cl_2 (18 ml) for 24 h, filtered, washed liberally with CH_2Cl_2, MeOH, and dried under vacuum (r.t., 0.5 Torr). The O-immobilized

hydroxylamine supports thus obtained were coupled with appropriate N-Fmoc-protected amino acids under standard conditions, (Grant (1992) *Synthetic Peptides. A User's Guide.* W.H. Freeman and Co.) and the resultant immobilized amino hydroxamic acids were employed in the preparation of the fused 2,4-pyrimidinedione hydroxamate derivatives in a manner similar to syntheses of fused 2,4-pyrimidinediones with terminal carboxylic functionalities (see the *General Procedures for Solid Phase Preparations of Fused 2,4-Pyrimidinediones*).

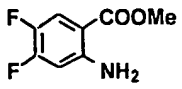
3-[(S)-1-Benzyl-1-hydroxamidomethyl]-2,4-(1H, 3H)-quinazolinedione.



The compound was prepared from FmocPhe-OH and the O-immobilized hydroxylamine support resin (made from the Sasrin resin as described above, see *General Procedure for Solid Phase Preparations of the Fused 2,4-Pyrimidinedione Hydroxamate Derivatives*). The crude reaction product was purified by preparative TLC (the major $FeCl_3$-positive spot; eluent : CH_2Cl_2 - MeOH 6:1). Mass-spectrum (m/z): 324 $(M-H)^-$.

Preparation of Intermediates for the Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones

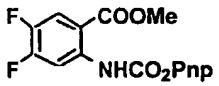
Methyl 4,5-Difluoroanthranilate



Triphosgene (0.65 g, 0.22 mmol) was added to 4,5-difluoroanthranilic acid (0.34 g, 0.2 mmol) in dry 1,4-dioxane (30 ml), and the mixture was stirred at rt. for 1 h. Solvent was slowly distilled off at ca. 50 °C, and the residue distributed between ethyl acetate (100 ml) and the pH 7 buffer (50 ml). The aqueous layer was extracted with more ethyl acetate

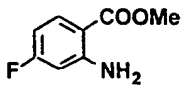
(2 x 50 ml), and combined organic layers washed with diluted aq. citric acid (pH ca. 4-5), water (30 ml), brine (50 ml), and dried ($MgSO_4$). Solvent was removed in vacuo to yield 0.33 g (83%) of 6,7-difluoroisatoic anhydride. The intermediate thus obtained (0.29 g, 1.45 mmol) was stirred with dry potassium carbonate (0.20 g, 1.45 mmol) in dry methanol (30 ml) for 1 h. Most of the solvent was removed in vacuo, and the residue distributed between ethyl acetate (100 ml) and the pH 7 buffer (50 ml). Aqueous layer was adjusted to pH ca. 7 with additional aq. HCl, and extracted with ethyl acetate (2 x 30 ml). Combined organic layers were washed with water (2 x 30 ml), brine (50 ml), and dried ($MgSO_4$). Solvent was removed in vacuo to yield 0.24 g (89%) of methyl 4,5-difluoroanthranilate. ^1H NMR in $CDCl_3$ (δ, ppm): 3.86 (s, 3 H), 5.72 (br. s, 2 H), 6.43 (m, 1 H), 7.66 (m, 1 H).

Methyl 2-(p-Nitrophenyl)carbamoyl-4,5-difluorobenzoate



Methyl 4,5-difluoroanthranilate (0.19 g, 1 mmol) in THF (1.5 ml) was added at ca. 0 °C to p-nitrophenyl chloroformate (0.21 g, 1.05 mmol) in pyridine (0.089 ml, 1.1 mmol) and dry acetonitrile (2.0 ml). The mixture was stirred at r.t. for 2 h. Solvent was removed in vacuo, and the residue triturated with ethyl ether (ca. 3 ml), filtered, washed with ether (2 x 2 ml), water (3 x 3 ml), hexanes (3 x 5 ml), and dried in vacuo. Yield 0.27 g (77%). ^1H NMR in $CDCl_3$ (δ, ppm): 3.98 (s, 3 H), 7.40 (d, J = 8.1 Hz, 2 H), 7.90 (m, 1 H), 8.30 (d, J = 8.1 Hz, 2 H), 8.38 (m, 1 H), 11.07 (s, 1 H).

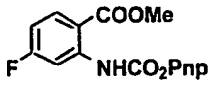
Methyl 4-Fluoroanthranilate Hydrochloride



Triphosgene (3.26 g, 11 mmol) was added to 4-fluoroanthranilic acid (1.55 g, 1.0 mmol) in dry 1,4-dioxane (50 ml), and the mixture was stirred at rt. for 1 h, and then 50 °C for ca. 30 min. Solvent was removed in vacuo, dry methanol (30 ml) added, followed by

potassium carbonate (4.42 g, 35 mmol). The mixture was stirred at r.t. for 30 min, and then 2.5 h at 50 °C. Inorganic material was filtered off, and the residue distributed between ethyl acetate (150 ml) and the pH 7 buffer (75 ml). Aqueous layer was extracted with ethyl acetate (2 x 50 ml). Combined organic layers were washed with water diluted aq. sodium bicarbonate (pH ca. 8; 2 x 50 ml), brine (50 ml), and dried ($MgSO_4$). The organic solution was concentrated in vacuo (to ca. 75 ml), and 1 M hydrogen chloride in ethyl ether (ca. 25 ml) added. The precipitated crystalline product was filtered off, washed with ethyl ether, hexanes, and dried in vacuo. Yield 1.00 g (49%). ^{1}H NMR in 5% CD_3OD in $CDCl_3$ (δ, ppm): 3.83 (s, 3 H), 6.55 (m, 1 H), 6.66 (m, 1 H), 7.88 (m, 1 H).

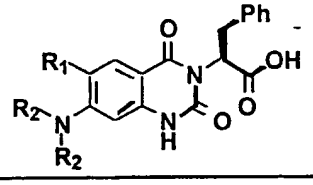
Methyl 2-(p-Nitrophenyl)carbamoyl-4-fluorobenzoate

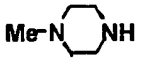
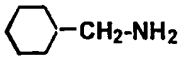
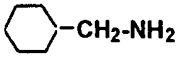
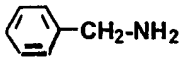


Pyridine (0.36 ml, 4.4 mmol) was added dropwise with stirring to the mixture of methyl 4-fluoroanthranilate hydrochloride (0.41 g, 2 mmol) and p-nitrophenylchloroformate (0.424 g, 2.1 mmol) in dry acetonitrile (7 ml). The mixture was stirred for 1.5 h at r.t. and evaporated. The residue was triturated with acetonitrile (1.5 ml), filtered, washed with ethyl ether (3 x 2 ml), acetonitrile (0.5 ml), ether (2 ml), and dried in vacuo. Yield 0.64 g (96%). ^{1}H NMR in $CDCl_3$ (δ, ppm): 3.97 (s, 3 H), 6.85 (m, 1 H), 7.42 (d, J = 8.1 Hz, 2 H), 8.11 (m, 1 H), 8.22 (m, 1 H), 8.29 (d, J = 8.1 Hz, 2 H), 11.23 (s, 1 H).

Table 3. Selected Examples 7-Substituted Quinazoline-2,4-diones Made Through Nucleophilic Aromatic Substitution

Table 2. 7-Fluoroquinazolinediones*



Entry #	R_1	Amine Substituent	HPLC purity, % (220nm)
1	H		80
2	F		97
3	F		97
4	F		95
5	F		96

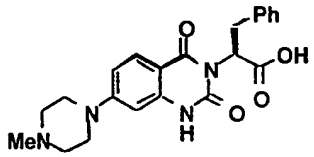
* See Fig. 6. ** Structures are in agreement with ^{1}H NMR and MS data.

General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones.

7-Fluoroquinazoline-2,4-dione Sasrin resin was prepared as we described previously in a prior patent application [Gordeev and Patel, Solid Phase and Combinatorial Syntheses of Fused 2,4-Pyrimidinediones, Serial No. 08/740,103]. The above resin (ca. 0.03 mmol) was agitated with an appropriate nucleophilic reagent (such as amine, 0.9 mmol) in N-methylpyrrolidine-2-one (1.25 ml) with or without organic base (such as triethylamine, diisopropylamine, tetraalkylguanidine, 1,8-diazabicyclo[5.4.0]undec-7-ene and alike) at 50-120 °C for 2-36 h (until the reaction was complete by gel-phase ^{19}F NMR of the resin; typically, 4-6 h for reactions of amines with 6,7-difluoroquinazolinedione resins; no addition of a base was required for preparation of compounds listed below and in the Table 2). This transformation with other nucleophilic reagents, such as thiols, alcohols, or NH-heterocycles is performed in polar organic solvents, such as pyridine, N-methylpyrrolidine-2-one, dimethylformamide, dimethylacetamide, acetonitrile,

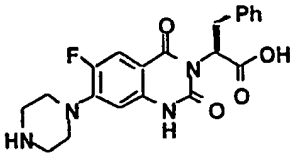
dimethylsulfoxide, sulfolan, tetrahydrofuran and alike, with or without addition of base (such as organic bases: triethylamine, diisopropylamine, tetraalkylguanidine, 1,8-diazabicyclo[5.4.0]undec-7-ene, or metal amides; or inorganic bases, such as alkali metal carbonates, sodium hydride, etc.), at 50-120 °C for 2-36 h. Resin after reaction was filtered, washed liberally with methanol and CD_2Cl_2, and dried in vacuo. Cleavage of the resin and product isolation were performed as described above.

3-[(S)-1-Benzyl-1-carboxymethyl]-7-[(4-methyl)-1-piperazino]-2,4-(1H,3H)-quinazolinedione



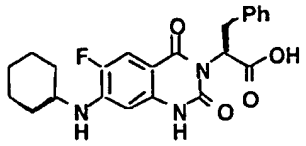
The product was prepared from Fmoc-Phe-Sasrin, methyl 2-(p-nitrophenyl)carbamoyl-4-fluorobenzoate, and 1-methylpiperazine as described above in the General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones. R_t 2.3 min. Mass-spectrum (m/z): 409 $(M+H)^+$.

3-[(S)-1-Benzyl-1-carboxymethyl]-6-fluoro-7-(1-piperazino)-2,4-(1H,3H)-quinazolinedione



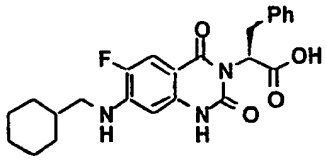
The product was prepared from Fmoc-Phe-Sasrin, methyl 2-(p-nitrophenyl)carbamoyl-4,5-difluorobenzoate, and piperazine as described above in the General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones. R_t 2.5 min. ^1H NMR in 5% CD_3OD in $CDCl_3$ (δ, ppm): 3.28-3.43 (m, 9 H), 3.45-3.58 (m, 1 H), 5.76 (m, 1 H), 6.49 (d, J = 6.9 Hz, 1 H), 7.00-7.18 (m, 5 H), 7.48 (d, J = 12.4 Hz, 1 H). Mass-spectrum (m/z): 413 $(M+H)^+$.

3-[(S)-1-Benzyl-1-carboxymethyl]-6-fluoro-7-cyclohexylamino-2,4-(1H,3H)-quinazolinedione



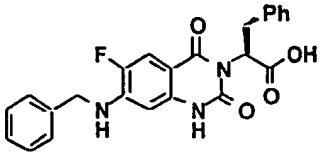
The product was prepared from Fmoc-Phe-Sasrin, methyl 2-(p-nitrophenyl)carbamoyl-4,5-difluorobenzoate, and cyclohexylamine as described above in the General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones. R_t 4.1 min. Mass-spectrum (m/z): 426 $(M+H)^+$.

3-[(S)-1-Benzyl-1-carboxymethyl]-6-fluoro-7-(cyclohexylmethyl)-amino-2,4-(1H,3H)-quinazolinedione



The product was prepared from Fmoc-Phe-Sasrin, methyl 2-(p-nitrophenyl)carbamoyl-4,5-difluorobenzoate, and (cyclohexylmethyl)amine as described above in the General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones. R_t 4.4 min. Mass-spectrum (m/z): 440 $(M+H)^+$.

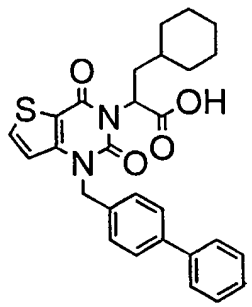
3-[(S)-1-Benzyl-1-carboxymethyl]-6-fluoro-7-benzylamino-2,4-(1H,3H)-quinazolinedione



The product was prepared from Fmoc-Phe-Sasrin, methyl 2-(p-nitrophenyl)carbamoyl-4,5-difluorobenzoate, and benzylamine as described above in the General Procedure for Solid Phase Synthesis of 7-Substituted Quinazoline-2,4-diones. R_t 3.8 min. Mass-spectrum (m/z): 434 $(M+H)^+$.

Although the forgoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practical. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

1-(4-Biphenyl)methyl-3-[(S)-1-cyclohexyl-1-carboxymethyl]-2,4-(1H,3H)-thieno[2,3-d]pyrimidine-2,4-dione.



The compound has been prepared from the FmocCha-Sasrin resin with 3-(p-nitrophenyl)carbamoyl-2-methoxycarbonylthiophene (Method A) of the General Procedures for Solid Phase Preparations of Fused 2,4-Pyrimidinediones. MS (m/z): 489 $[M-H]^-$.

Preparation of 1,200 Member Library of Quinazoline-2,4-diones.

The library (FIG. 8) was prepared using experimental protocols analogous to those employed for preparation of the individual compounds, as described above in Method A. The basic strategy for the synthesis of the library involved three steps. Sixty N^1-unsubstituted quinazoline-2,4-diones immobilized on Sasrin resin were prepared. These quinazoline-2,4-dione intermediates were then alkylated. Finally, the N^1-alkylated quinazoline-2,4-dione products were cleaved from the solid support in preparation for biological testing.

A. Preparation of 60 N^1-Unsubstituted Quinazoline-2,4-dione Intermediates.

Sixty individual N^1-unsubstituted quinazoline-2,4-diones immobilized on Sasrin resin were prepared from 15 amino acids and 4 methyl anthranilate derived reagents (for structures of the building blocks, see Fig. 8) according to the Method A of the General Procedures for Solid Phase Preparations of N^1-Unsubstituted Fused 2,4-Pyrimidinediones. Each respective N-Fmoc-protected amino acid resin (ca. 0.2-0.3 mmol) was deprotected with 20% piperidine in dimethylformamide (5-10 mL) for 30 min. The resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. The selected amine resin was suspended with one of four isocyanate or p-nitrophenylcarbamate methyl anthranilate reagents (see Fig. 8, 0.8-2 mmol) in dimethylformamide (4-8 mL; for reaction with isocyanate) or 10% pyridine in dimethylformamide (4-8 mL; for reactions with activated carbamates), and agitated at room temperature until a negative ninhydrine test indicated absence of a free amine (typically, reaction with isocyanate was performed for ca. 3 h, and reactions with p-nitrophenylcarbamate were performed overnight). The resultant urea resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. Immobilized urea derivatives thus obtained were further cyclized into quinazolinediones by agitation at ca. 50-60°C with 5% tetramethylguanidine in dimethylformamide (5-10 mL) overnight. The resin was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. Prior to further manipulations, ca. 20-30 mg of each quinazoline-2,4-dione resin was cleaved with 1-3% TFA in DCM, and the product analyzed by ^{1}H-NMR and MS. This procedure was repeated for each of the 15 immobilized amino acids and 4 methyl anthranilate reagents to generate 60 individual immobilized N^1-unsubstituted quinazoline-2,4-diones.

B. Alkylations of 60 N^1-Unsubstituted Quinazoline-2,4-dione Intermediates With 20 Alkylating reagents to Produce 1,200 Member Quinazoline-2,4-dione Library. Four N^1-substituted quinazoline-2,4-dione intermediates were individually suspended in a mixture of carbon tetrachloride and N-methylpyrrolidine-2-one (ca. 1:5, ca. 10 mL). Each of the 4 suspensions was distributed into 20 wells of a 96-well teflon reaction plate supplied with bottom filter frits. Solvent was drained from the plate, and the plate was

further dried under vacuum. The plate bottom was sealed with a teflon or rubber seal. The plate-loaded quinazoline-2,4-dione intermediates were individually alkylated with 20 alkyl halides (see FIG. 8) according to the Method A of the General Procedures for Solid Phase Preparations of N^1-Alkylated Fused 2,4-Pyrimidinediones: Stock solutions of each of the 20 alkylating reagents (ca. 10-15 mmol) in a mixture of dimethylsulfoxide and N-methylpyrrolidine-2-one (1:1, ca. 20 mL, except for alkylating reagents employed as hydrochloride salts, where an equivalent amount of tetrabutylammonium iodide (10-15 mmol) was also added), or N-methylpyrrolidine-2-one (in all other cases; ca. 20 mL) and tetramethylguanidine (ca. 10-15 mmol; except for hydrochloride salt reagents, where an extra equivalent of the base was employed), were distributed individually (ca. 1 mL in each well) into each of the 80 wells of the reaction plate. The plate was sealed with a teflon/rubber seal, and agitated by vortexing for approximately 48 h. The plate was filtered, washed liberally with dimethylformamide, MeOH, and CH_2Cl_2, and dried under vacuum. Eighty different alkylated quinazoline-2,4-dione resins were therefore prepared on the 96-well plate. This protocol was repeated for 15 different plates to produce 1200 individual, alkylated quinazoline-2,4-dione resins. Each plate was placed on a receptacle 96 well plate, and 2-3% trifluoracetic acid in dichloromethane (ca. 2 ml) was added. The receptacle plates were dried under vacuum and submitted for biological testing. Where amino acids with protected side chains were used, an additional deprotection step was performed directly in a receptacle plate with 40% trifluoroacetic acid in dichloromethane containing ca. 3-5% triethylsilane (ca. 1 ml in each well; for Ser, Tyr, Glu, Gln, Lys, Asp, Asn) or with 40% trifluoroacetic acid in dichloromethane containing anisole 3-5% (ca. 1 ml in each well; for Trp).

Assay Protocol for beta-Lactamase Inhibition.

The lactamase (20-120 ng/mL) was incubated with a potential inhibitor with 1% DMSO in 50 mM potassium phosphate buffer, pH 7.0, with 0.005% Brij-35 for 30 min at room temperature. 100 μM of nitrocefin was then added to the reaction mixture and the hydrolysis of the nitrocefin was monitored by measuring the absorption increase at 490 nm. Inhibition of the potential compounds was calculated by comparing the rate of

absorption increase with the control sample which containing the identical mixture except inhibitors. The IC_{50}, was obtained by fitting the inhibition data into a standard 2-parameter IC_{50} equation with a non-linear least-square fitting program (DeltaGraph).

Assay Protocol for Antimicrobial Activity.

Minimum inhibitory concentrations (MICs) were determined using the microdilution method in 96-well format plates. Compounds were suspended in DMSO at 5 or 10 mg/ml and stored at 4°C until used. They were diluted in Mueller-Hinton Broth (MHB) or Trypticase Soy Broth (TSB) and used for MIC determination. The range of concentrations tested was 64-0.0625 μg/ml final concentration using a two-fold dilution system.

The inoculum was prepared from cells grown on Trypticase Soy Agar (TSA) and incubated overnight at 35 °C, 5 to 10 colonies were used to inoculate MHB or TSB broths, and the culture was incubated overnight at 35°C. The overnight culture was diluted 1:10, incubated for one hour at 35°C, diluted to the appropriate inoculum size and applied to the wells containing broth and test compound. Inoculum sizes were $1x10^5$ to $5x10^5$ CFU/ml. Strains used were *P. aeruginosa* VPAE1001, E. *faecium V* VEFA1001, *E. faecium* VanA VEFA1002, *S. aureus* VSAU1003, and *S. aureus* MRSA VSAU1004.

Plates were incubated at 35°C for 48 hours and MIC were recorded after 18 hours of incubation, for bacteria, and 48 for yeasts. MIC was defined as the lowest concentration of compound that does not produce visible growth after incubation.

Selected Bioactive Compounds

From the 1200 member 2,4-quinazolinedione library described above, 5 compounds possessing an IC_{50} for β-lactamase inhibition in the range of 1.3 to 183 μm and an MIC in the range of 3.12 to >50 μg/ml were found. These compounds are shown in Fig. 9: VRC0056-01, VRC0090-01, VRC0057-01, VRC0058-01, and VRC0059-01.

Analogue Synthesis And Screening

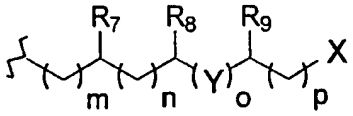
Based on the positive results observed from the library screening, a number of analogues of the bioactive quinazoline-2,4-diones were synthesized using Method A of the *General Procedures for Solid Phase Preparations of Fused 2,4-Pyrimidinediones*. These analogues were subsequently assayed. From this set of compounds, 9 additional compounds possessing an IC_{50} for β-lactamase inhibition in the range of 1.3 to183 μm and an MIC in the range of 3.12 to >50 μg/ml were identified. These compounds are shown in FIG. 9: VRC0094-01, VRC0091-01, VRC0095-01, VRC0092-01, VRC0096-01, VRC0093-01, VRC0097-01, VRC0098-01, and VRC00127-01.

CLAIMS

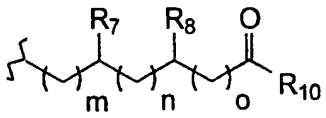
1. A combinatorial library comprising compounds of the structure:

P-R

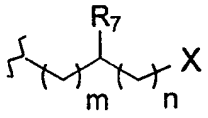
where P is a fused 2,4-pyrimidinedione selected from the group consisting of pyrimidopyrimidinediones, 2,4-pteridinediones, pyrimidopyridazinediones and azolopyrimidinediones, and where R is a substituted alkyl chain at the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and further where m, n, o, and p vary independently from 0 to 4,



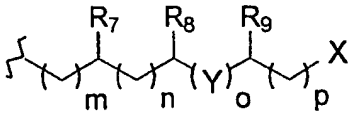
where R_7 and R_8 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m, n, and o vary independently from 0 to 4, and



where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m and n vary independently from 0 to 4.

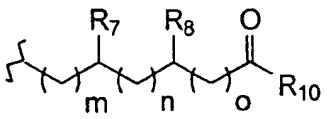
2. The combinatorial library according to claim 1, where P is selected from the group consisting of pyrimidopyrimidinediones and azolopyrimidinediones.

3. The combinatorial library according to claim 1, where R is a substituted alkyl chain of the structure:



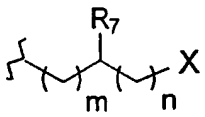
where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and further where m, n, o, and p vary independently from 0 to 4.

4. The combinatorial library according to claim 1, where R is a substituted alkyl chain of the structure:



where R_7 and R_8 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m, n, and o vary independently from 0 to 4.

5. The combinatorial library according to claim 1, where R is a substituted alkyl chain of the structure:



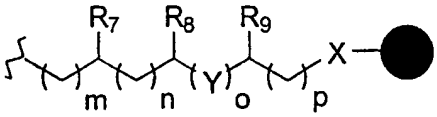
where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m and n vary independently from 0 to 4.

6. A combinatorial library comprising compounds of the structure:

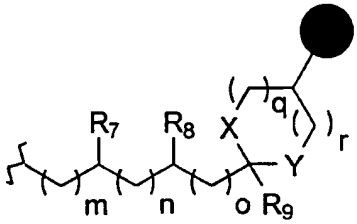
P-R-●

where P is a fused 2,4 pyrimidinedione selected from the group consisting of 2,4-pteridinediones, pyridopyrimidinediones, pyrimidopyridazinediones, 2,4-

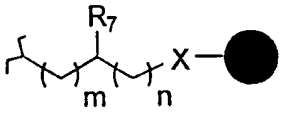
quinazolinediones and azolopyrimidinediones, and where R is a substituted alkyl chain on the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and further where m, n, o, and p vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl,



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where X and Y are independently selected from the group consisting of O and S; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and



where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and a natural amino acid side chain; and where

X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl;

and ● is a solid support.

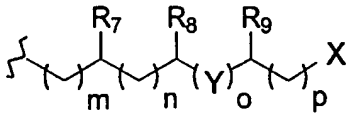
7. The combinatorial library according to claim 6, where P is selected from the group consisting of pyrimidopyrimidinediones and azolopyrimidinediones.

8. The combinatorial library according to claims 1 or 6, where P is an azolopyrimidinedione.

9. A method for synthesizing a combinatorial library comprising compounds of the structure:

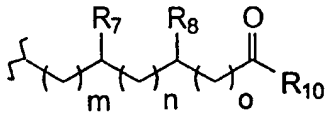
P-R

where P is a fused 2,4-pyrimidinedione selected from the group consisting of pyrimidopyrimidinediones, 2,4-pteridinediones, pyrimidopyridazinediones, pyridopyrimidinediones, 2,4-quinazolinediones and azolopyrimidinediones, and where R is a substituted alkyl chain at the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:

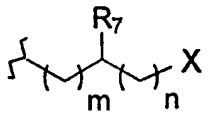


where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2,

NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and further where m, n, o, and p vary independently from 0 to 4,



where R_7 and R_8 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m, n, and o vary independently from 0 to 4, and



where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m and n vary independently from 0 to 4, comprising the steps of

(a) immobilizing a substrate on a solid support, where the substrate is selected from the group consisting of amines, isocyanate, activated carbamate and moieties capable of being functionalized to form an amine;

(b) optionally functionalizing the substrate to form an amine;

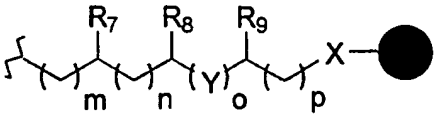
(c) reacting the substrate with an amine where the substrate is an isocyanate or activated carbamate or an isocyanate or activated carbamate where the substrate is an amine; and

(c) cleaving the resulting solid support bound 2,4-pyrimidinediones from the solid support.

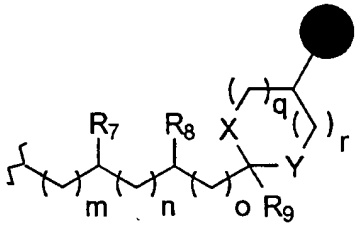
10. A method for synthesizing a combinatorial library comprising compounds of the structure:

P-R-●

where P is a fused 2,4 pyrimidinedione selected from the group consisting of pyrimidopyrimidinediones, 2,4-pteridinediones, pyrimidopyridazinediones, pyridopyrimidinediones, 2,4-quinazolinediones and azolopyrimidinediones, and where R is a substituted alkyl chain on the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:

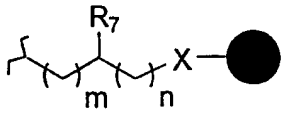


where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and further where m, n, o, and p vary independently from 0 to 4; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl,



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where X and Y are independently selected from the group consisting of O and S; and

where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and



where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and a natural amino acid side chain; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and further wherein ● is a solid support, comprising the steps of

(a) immobilizing a substrate on a solid support, where the substrate is selected from the group consisting of amines, isocyanate, activated carbamate and moieties capable of being functionalized to form an amine;

(b) optionally functionalizing the substrate to form an amine;

(c) reacting the substrate with an amine where the substrate is an isocyanate or activated carbamate or an isocyanate or activated carbamate where the substrate is an amine; and

(c) cleaving the resulting solid support bound 2,4-pyrimidinediones from the solid support.

11. The method according to claims 9 or 10, where the fused 2,4-pyrimidinedione is selected from the group consisting of pyrimidopyrimidinediones, 2,4-pteridinediones, pyrimidopyridazinediones and azolopyrimidinediones.

12. The method according to claims 9 or 10, where the substrate immobilized on the solid support is an amine.

13. The method according to claims 9 or 10, where the substrate immobilized on the solid support is an isocyanate.

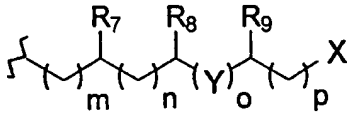
14. The method according to claims 9 or 10, where the substrate immobilized on the solid support is an activated carbamate.

15. The method according to claims 9 or 10, where as an additional step a fluoride substituent is displaced in a nucleophilic aromatic substitution reaction.

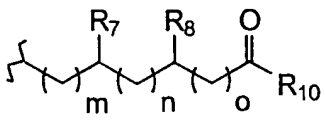
16. A combinatorial library comprising compounds of the structure:

P-R

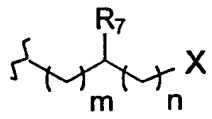
where P is a fused 2,4-pyrimidinedione selected from the group consisting of 2,4-quinazolinediones and pyridopyrimidinediones and where R is a substituted alkyl chain at the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and further where m, n, o, and p vary independently from 0 to 4,



where R_7 and R_8 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{10}$, and an amino acid side chain; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m, n, and o vary independently from 0 to 4, and



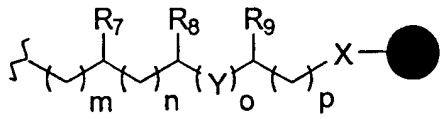
where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where X is selected from the group consisting of CO_2H, CO_2R_{10}, $C(O)R_{10}$, SH, OH, NH_2, NHR_{10}, C(O)NHOH, and $C(O)NHR_{10}$; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl and heteroaryl; and where m and n vary independently from 0 to 4.

17. The combinatorial library according to claim 16, where the fused 2,4-pyrimidinedione is a 2,4-quinazolinedione.

18. A combinatorial library comprising compounds of the structure:

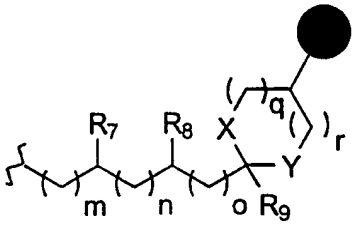
P-R-●

where P is a 2,4-quinazolinedione and wherein R is a substituted alkyl chain on the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:

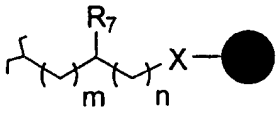


where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain;

and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and further where m, n, o, and p vary independently for 0 to 4; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl,



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where X and Y are independently selected from the group consisting of O and S; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and



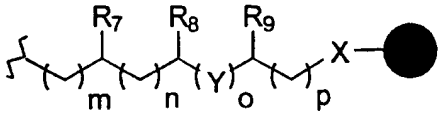
where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and a natural amino acid side chain; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl,

and ● is a solid support.

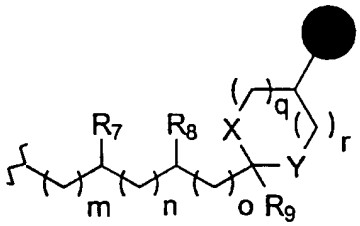
19. A method for synthesizing a combinatorial library comprising compounds of the structure:

P-R-●

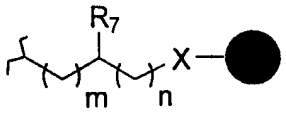
where P is a fused 2,4 pyrimidinedione selected from the group consisting of 2,4-quinazolinediones and pyrimidopyrimidinediones, and where R is a substituted alkyl chain on the 3-position of the fused 2,4-pyrimidinedione selected from the group consisting of:



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and an amino acid side chain; and where Y is selected from the group consisting of alkyl, aryl, O, NH, and NR_{10}; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and further where m, n, o, and p vary independently for 0 to 4; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl,



where R_7, R_8, and R_9 are independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, =O, $C(O)R_{11}$, and an amino acid side chain; and where X and Y are independently selected from the group consisting of O and S; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and



where R_7 is independently selected from the group consisting of H, alkyl, aryl, OH, NH_2, NHR_{10}, $NR_{10}R_{11}$, SH, SR_{10}, =O, $C(O)R_{11}$, and a natural amino acid side chain; and where X is selected from the group consisting of CO_2, CO, S, O, NH, NR_{10}, C(O)NHO, and $C(O)NR_{10}$; and where m, n, and o vary independently from 0 to 4 and q and r vary independently from 0 to 2; and where R_{10} and R_{11} are independently selected from the group consisting of alkyl, aryl, and heteroaryl, and further wherein ● is a solid support, comprising the steps of

(a) immobilizing a substrate on a solid support, where the substrate is selected from the group consisting of amines, isocyanate, activated carbamate and moieties capable of being functionalized to form an amine;

(b) optionally functionalizing the substrate to form an amine;

(c) reacting the substrate with an amine where the substrate is an isocyanate or activated carbamate or an isocyanate or activated carbamate where the substrate is an amine; and

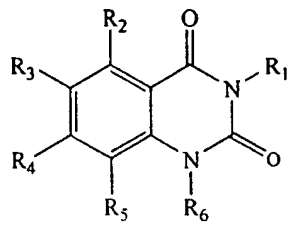
(c) cleaving the resulting solid support bound 2,4-pyrimidinediones from the solid support.

20. A method for screening the library according to claims 1 or 16 comprising:

a) contacting the fused 2,4-pyrimidinedione with a targeted receptor or enzyme under conditions conducive to specific binding; and

b) isolating the fused 2,4-pyrimidinedione that specifically binds to the targeted receptor or enzyme.

21. A bioactive compound having antimicrobial activity, said compound having the formula



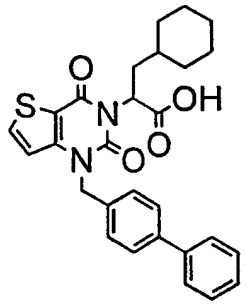
where

R_1 is an amino acid derived group;

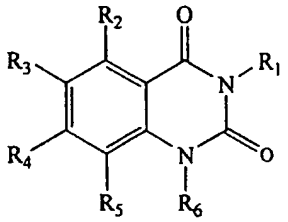
R_2-R_5 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, and electron withdrawing substituents; and

R_6 is selected from the group consisting of hydrogen, alkyl, aryl, and heteroaryl substituents.

22. A bioactive compound having antimicrobial activity, said compound having the formula



23. A bioactive compound having β-lactamase inhibitory activity, said compound being of the formula



where

R_1 is an amino acid derived group;

R_2-R_5 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, and electron withdrawing substituents; and

R_6 is selected from the group consisting of hydrogen, alkyl, aryl, and heteroaryl substituents.

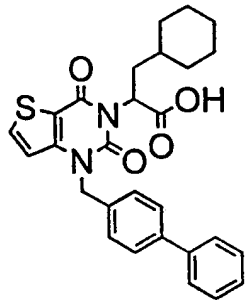
24. The compound according to claims 21 or 23, where R_1 is selected from the group consisting of α-amino acid groups, α-amino acid derivative groups, β-amino acid groups, and β-amino acid derivative groups.

25. The compound according to claims 21 or 23, where R_1 is selected from the group consisting of α-amino acid groups and α-amino acid derivative groups.

26. The compound according to claim 25, where R_6 is selected from the group consisting of alkyl and aryl substituents.

27. The compound according to claim 25, where R_6 is an alkyl substituent.

28. A bioactive compound having β-lactamase inhibitory activity, said compound having the formula



29. A pharmaceutical composition for antimicrobial use that comprises a compound according to claims 21 or 22.

30. A pharmaceutical composition for inhibiting β-lactamase activity that comprises a compound according to claims 23 or 28.

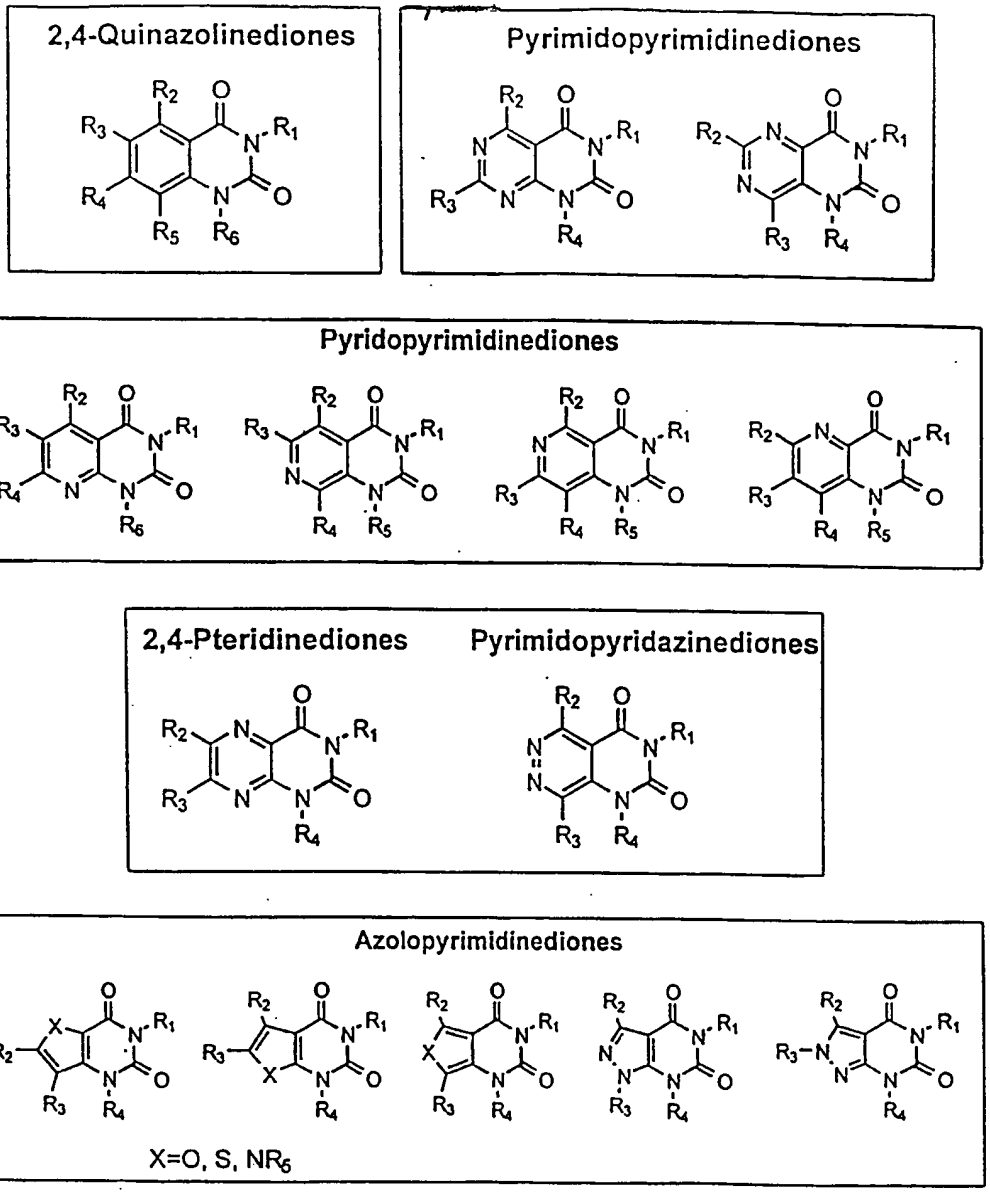

2,4-Quinazolinediones
Pyrimidopyrimidinediones
Pyridopyrimidinediones
2,4-Pteridinediones
Pyrimidopyridazinediones
Azolopyrimidinediones
X=O, S, NR5

FIGURE 1

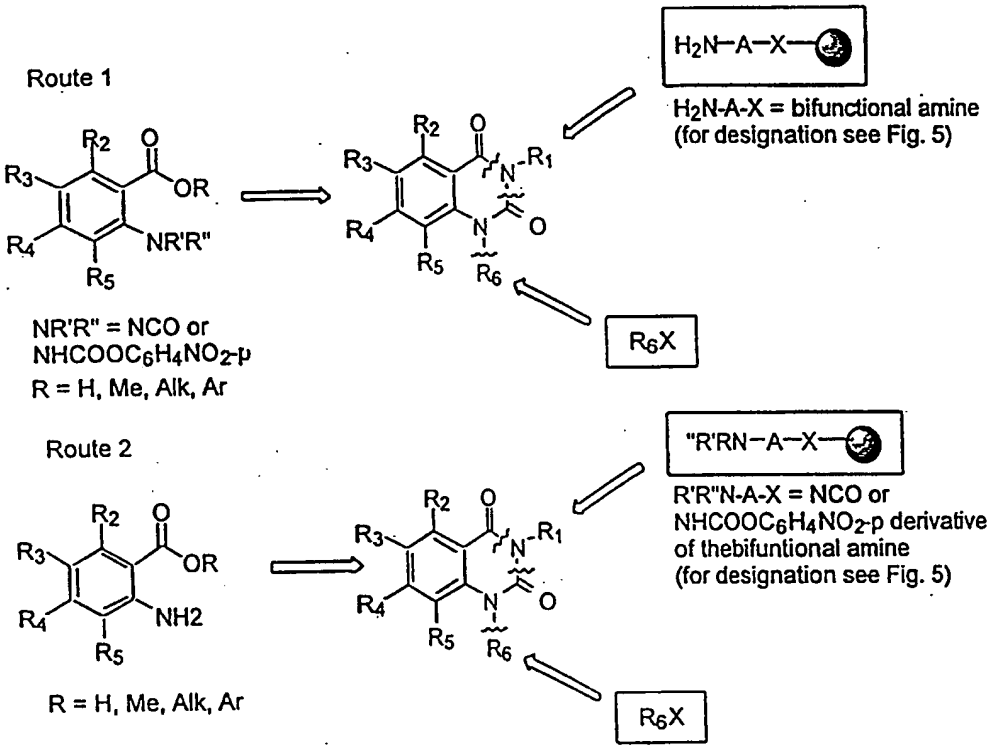

Route 1
H_2N–A–X
H_2N-A-X = bifunctional amine
(for designation see Fig. 5)
NR'R" = NCO or
$NHCOOC_6H_4NO_2$-p
R = H, Me, Alk, Ar
R_6X
Route 2
"R'RN–A–X
R'R"N-A-X = NCO or
$NHCOOC_6H_4NO_2$-p derivative
of thebifuntional amine
(for designation see Fig. 5)
R = H, Me, Alk, Ar
R_6X

FIGURE 2

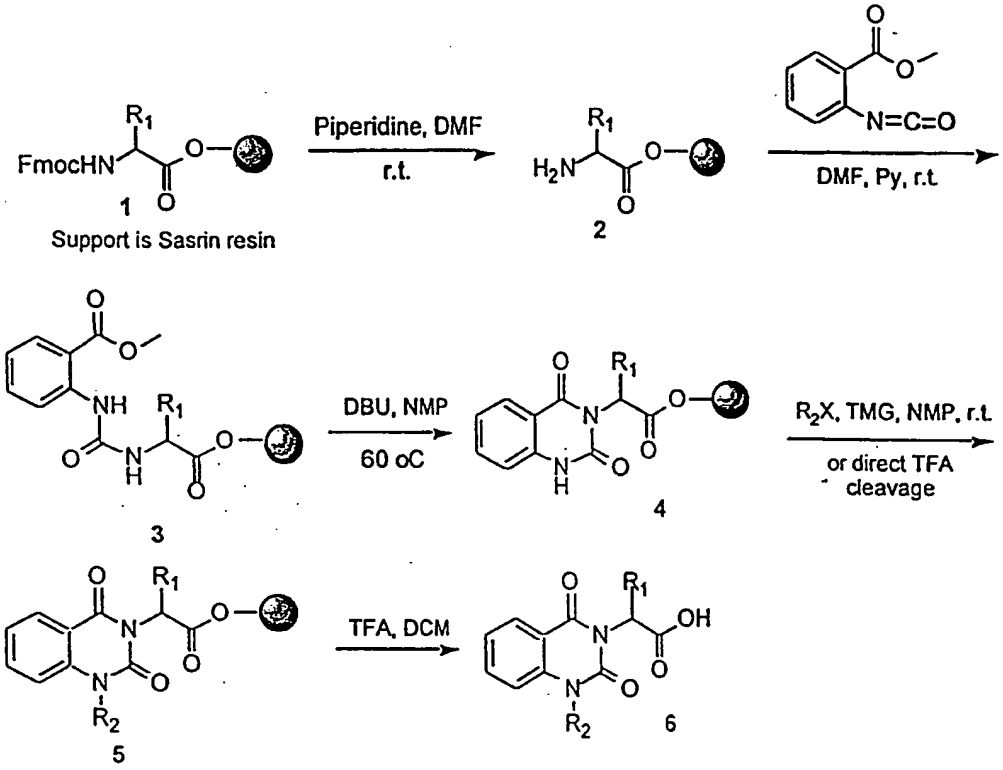

FmocHN
R1
O
1
Support is Sasrin resin
Piperidine, DMF
r.t.
H2N
2
N=C=O
DMF, Py, r.t.
NH
N
H
3
DBU, NMP
60 oC
4
R2X, TMG, NMP, r.t.
or direct TFA
cleavage
TFA, DCM
OH
R2
5
6

FIGURE 3

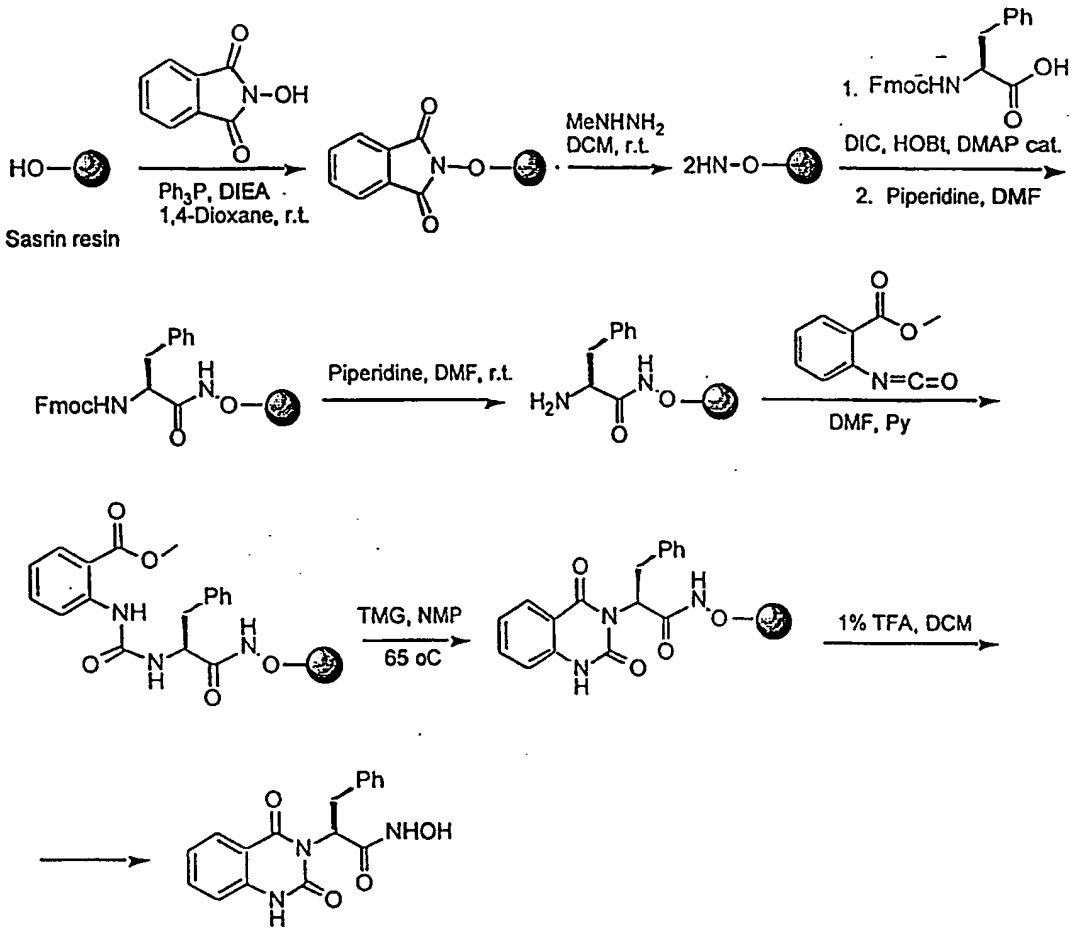
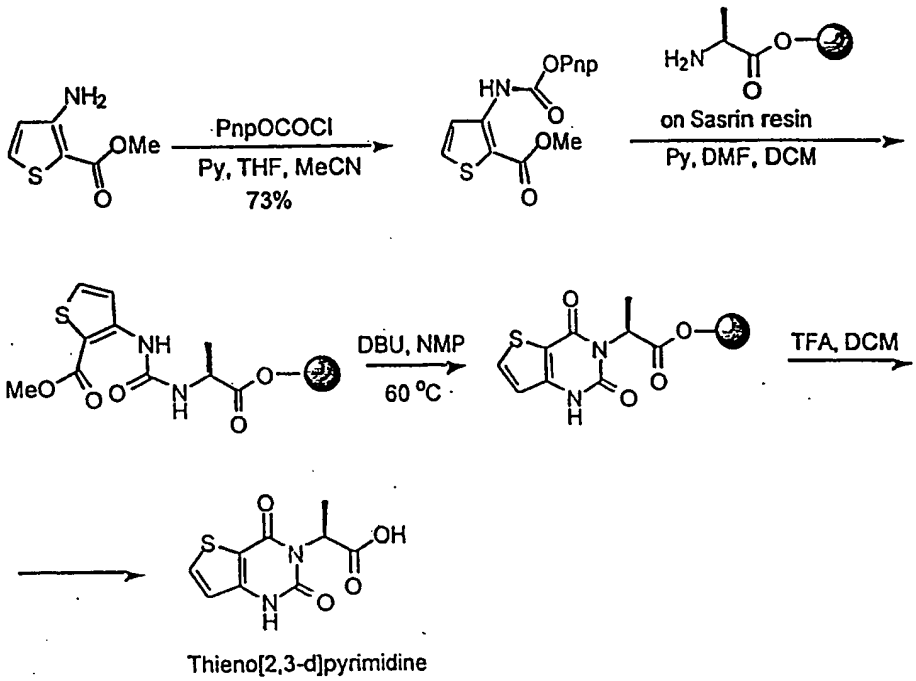

HO
Sasrin resin
N-OH
Ph3P, DIEA
1,4-Dioxane, r.t.
MeNHNH2
DCM, r.t.
2HN-O
1. FmocHN
OH
DIC, HOBt, DMAP cat.
2. Piperidine, DMF
FmocHN
Piperidine, DMF, r.t.
H2N
N=C=O
DMF, Py
NH
TMG, NMP
65 oC
1% TFA, DCM
NHOH

FIGURE 4



Thieno[2,3-d]pyrimidine

FIGURE 5

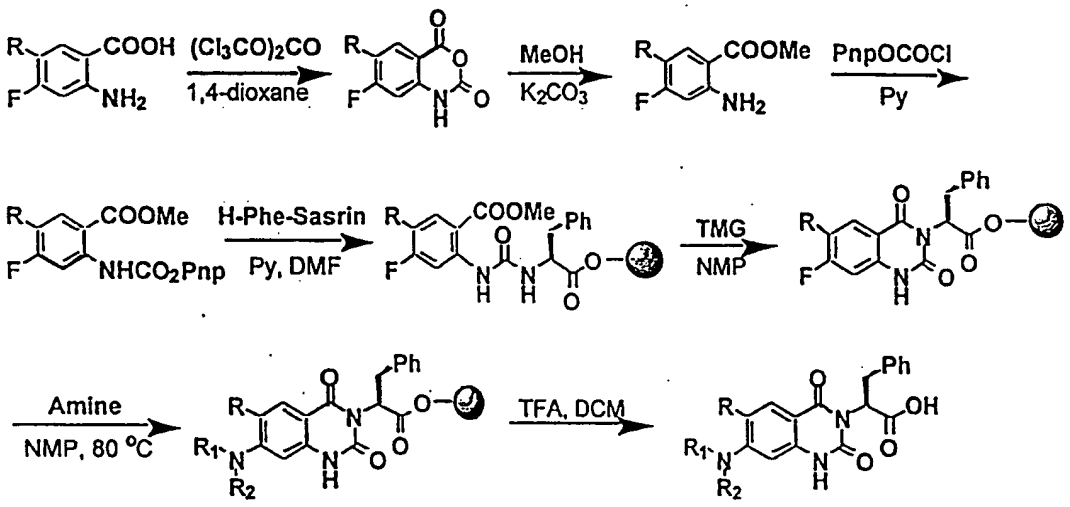
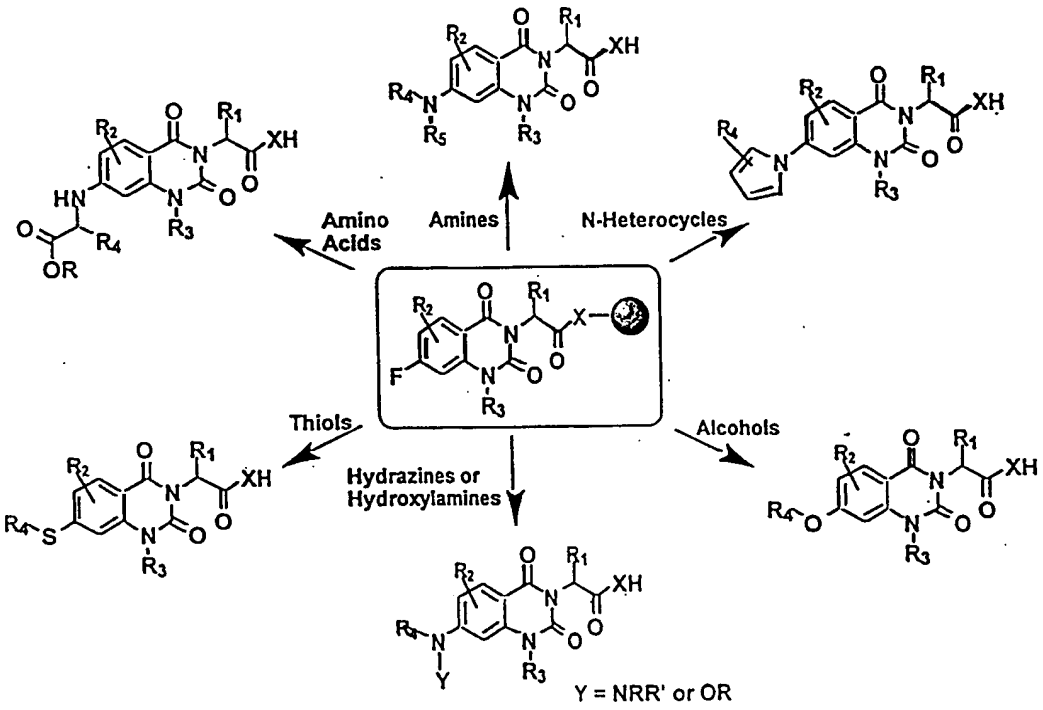

COOH
$(Cl_3CO)_2CO$
1,4-dioxane
MeOH
K_2CO_3
COOMe
NH_2
PnpOCOCl
Py
$NHCO_2Pnp$
H-Phe-Sasrin
Py, DMF
Ph
TMG
NMP
Amine
NMP, 80 °C
TFA, DCM
OH

FIGURE 6



R_2 can represent from 1 to 3 (different) substituent(s)

FIGURE 7

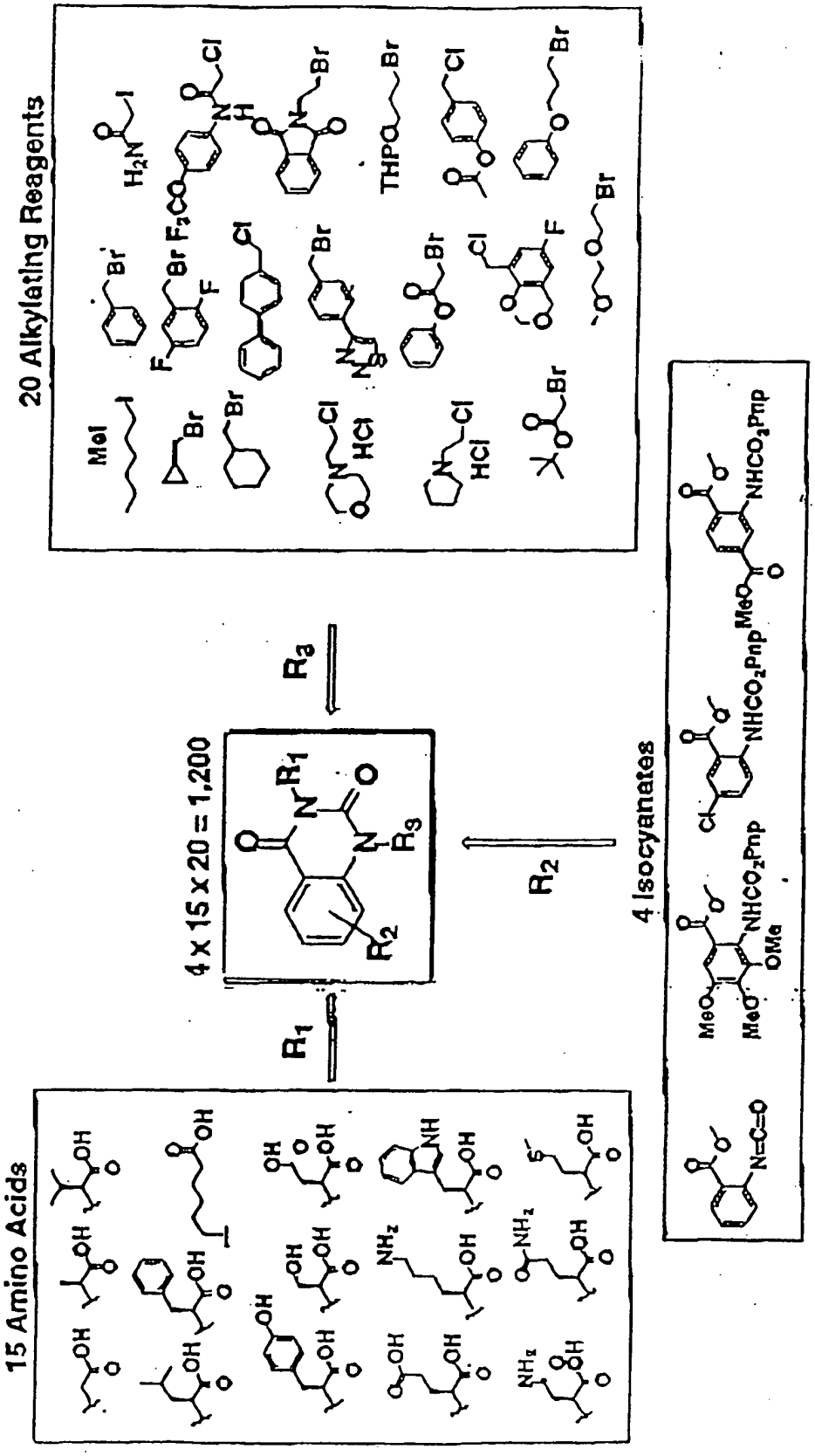
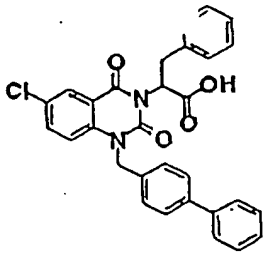
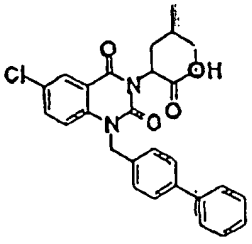

20 Alkylating Reagents
15 Amino Acids
4 x 15 x 20 = 1,200
4 Isocyanates
R1
R2
R3

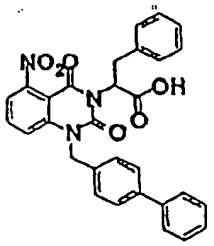
Figure 8



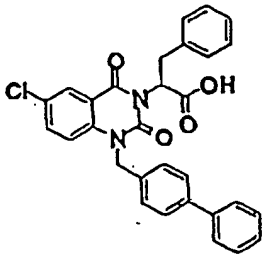
VRC0056-01



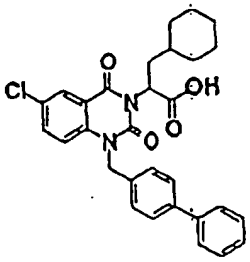
VRC0090-01



VRC0094-01



VRC0057-01



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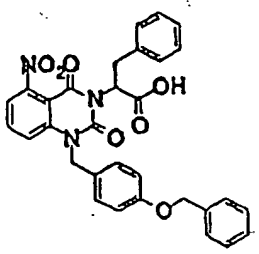
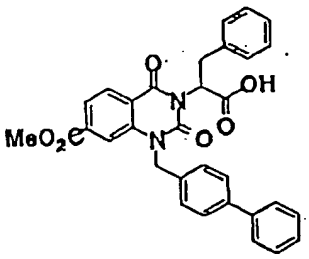
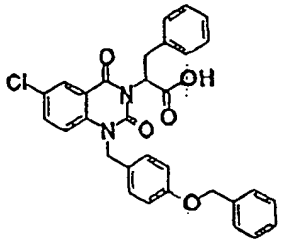


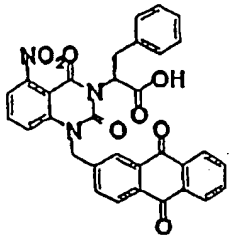
VRC0095-01



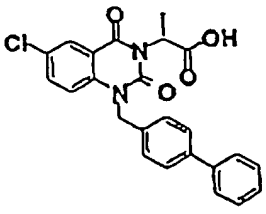
VRC0058-01



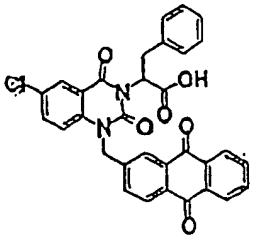
VRC0092-01



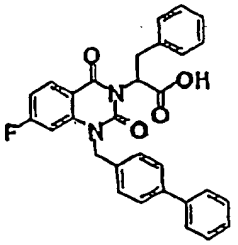
VRC0096-01



VRC0059-01



VRC0093-01



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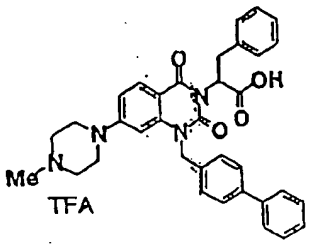
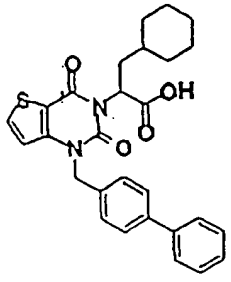


VRC0098-01



VRC00127-01

Figure 9

INTERNATIONAL SEARCH REPORT

Intern. nal Application No
PCT/US 97/19483

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D239/96 C07D513/04 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 114, no. 11, 18 March 1991 Columbus, Ohio, US; abstract no. 102035e, page 756; column 1; XP002061888 see abstract & JP 02 225 485 A (TAIHO PHARM. CO LTD) 7 September 1990 ---	22,28
A	EP 0 640 606 A (TAKEDA CHEMICAL INDUSTRIES, LTD.) 1 March 1995 ---	22,28
A	WO 96 24597 A (TAKEDA CHEMICAL INDUSTRIES) 15 August 1996 --- -/--	22,28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1998	30.04.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hartrampf, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna..ial Application No
PCT/US 97/19483

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. MED. CHEM., vol. 34, no. 4, April 1991, pages 1492-1503, XP002023720 ---	22,25-28
A	TETRAHEDRON LETT., vol. 37, no. 39, 1996, pages 7031-7034, XP004030817 cited in the application ---	22,25-28
A	HETEROCYCLES, vol. 36, no. 6, June 1993, pages 1305-1314, XP002061885 cited in the application ---	25-27
A	US 4 521 420 A (MAURER, F. ET AL.) 4 June 1985 -----	25-27

3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/19483

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/19483

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 1-21,23,24,29 and 30

because they relate to subject matter not required to be searched by this Authority, namely:

The definitions of residues R, R1, R2, R3, R4, R5 and R6 are too general and/or encompass too broad a range of different chemical groups only partly supported by the examples given in the description: in a non exhaustive preliminary search at least 51 documents have been found which destroy the novelty of claim 1 and at least 51 documents have been found that destroy the novelty of claim 16. The vast number of theoretically conceivable compounds precludes a complete comprehensive search. Guided by the inventive concept as disclosed in the description of the present application the search has been limited to claims 22, 25, 26, 27 and 28, cf. Articles 6 and 15 PCT, Rule 33 PCT and the PCT Search Guidelines, chapter III, 3.6, 3.7 and 3.12 and chapter VIII, 2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/19483

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 640606 A	01-03-95	CA 2130859 A	27-02-95
		CN 1106663 A	16-08-95
		FI 943912 A	05-06-95
		HU 71116 A	28-11-95
		JP 8073467 A	19-03-96
		NO 943146 A	27-02-95
		FI 950021 A	30-12-95
WO 9624597 A	15-08-96	AU 2223995 A	10-11-95
		AU 4632796 A	27-08-96
		BR 9501736 A	14-11-95
		CA 2186124 A	26-10-95
		CA 2211969 A	15-08-96
		CZ 9603028 A	14-05-97
		EP 0756599 A	05-02-97
		EP 0808317 A	26-11-97
		FI 964195 A	17-12-96
		HU 76320 A	28-08-97
		JP 9169768 A	30-06-97
		JP 8295693 A	12-11-96
		WO 9528405 A	26-10-95
		NO 964434 A	18-10-96
		PL 316796 A	17-02-97
US 4521420 A	04-06-85	DE 3228871 A	09-02-84
		EP 0101902 A	07-03-84
		JP 59044366 A	12-03-84